

#99
SOY BEAN OIL HYDROGENATED VOL I

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soy Bean Oil Hydrogenated-Volume I

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Table of Contents

Summary

CHEMICAL INFORMATION

	Page
I. Nomenclature	1
II. Empirical Formula	2
III. Structural Formula	11
IV. Molecular Weight	11
V. Specifications	11
VI. Description	12
VII. Analytical Methods	19
VIII. Occurrence	26

BIOLOGICAL DATA

I. Acute Toxicity	27
II. Short Term Studies	28
Direct Toxicity	28
Nutritional Imbalance	29
EFA Deficiency	30
Vitamin E or Antioxidant Deficiency	33
Autoxidation and Peroxidation Effects	34
III. Long Term Studies	38
IV. Special Studies	40

BIOCHEMICAL ASPECTS

I. Breakdown	46
II. Absorption - Distribution	55
III. Metabolism and Excretion	58
IV. Effects on Enzymes and Other Biochemical Parameters	61
V. Drug Interactions	68
VI. Consumer Exposure	68

SUMMARY

Description and Specifications

Soybean oil is the oil expressed from the seeds of the soybean or soya bean (Glycine max (L.) or G. soja); these are all synonyms. Its composition varies according to soil, climate, and other conditions of plant growth (022,125,134). In general the fatty acids (FA) are present mostly as triglycerides; about one-half of the FA are polyunsaturated (PUFA) and 90% of the PUFA are diene. Soybean oil also contains small amounts of sterols including cholesterol.

The purpose of hydrogenation is to elaborate a stable, palatable product of suitable plasticity; chemically, the aim is to part-saturate polyene, but not monoene, chains (013). Both the process and the products of hydrogenation are variable, and hydrogenated soybean oils are never more than partly hydrogenated (081,167,245,305). The proportion of double bonds left unsaturated is reflected by the iodine number. Hydrogenation also changes the FA composition in other ways -- not very predictably in most cases (013,167,305). Manufacturers tend to patent particular procedures to protect products with particular handling characteristics (046,295,299,314,371). So hydrogenated soybean oils include a wide range of chemically different oils that vary according to source and subsequent treatment (166,250). No complete analysis of any one sample was found in the literature.

The most important food uses of hydrogenated soybean oil in the United States are in margarines and shortenings (Tables 14 & 15). However, there is no standard of identity for hydrogenated soybean oil, and the nearest approach is the Codex Alimentarius Recommended International Standard for Edible Soya Bean

are "all only potential effects" that can be "controlled by the use to which the oil is put."

Theoretically peroxides can give rise to epoxides, which can be carcinogenic (153). No studies were found to suggest that this might occur in vitro or in vivo with hydrogenated soybean oil.

Long Term Studies

No long term studies were found. A series of patients (47 years average) fed soybean oil, not hydrogenated, showed no difference from the controls in the relapse rate for myocardial infarctions (351). The controversial question of the general relationships of saturated/unsaturated fats, cholesterol, and atherogenesis was considered to be outside the scope of a monograph restricted to hydrogenated soybean oil; some reports involving soybean oil are mentioned in the section on Metabolism.

A review by Scott (367) on the geography and incidence of selenium deficiency in the United States is summarized in view of the current debate about whether excesses of selenium are carcinogenic. Scott concludes otherwise, and emphasizes that selenium is probably an essential nutrient in the range 0.1-0.3 ppm, although toxic in the range 2-10 ppm. Absence of selenium affects the activity of vitamin E, and the bioavailability of selenium in soybean meal feeds is relatively low (367).

Special Studies

No tolerances have been set by the Food and Drug Administration for levels of residues from chlorinated hydrocarbon pesticides in edible oils; such tolerances have been set, however for seeds such as soybeans (147). Gooding et al. (147) and Smith et al. (378) concluded that all such residues would be removed by normal refining, hydrogenation, and post-hydrogenation treatment of the oils. Stout

Breakdown and
Metabolism

et al. (390), however, have denied this claim and have pointed to some of the data of Smith et al. (378) as supporting their denial. The debate revolves round the temperature of final deodorization, and is not yet concluded.

Methods for detecting accidental contamination of oils by polychlorinated biphenyls remain imperfect (043), and no reports on their elimination were found. Oxidation results in "off" flavors (082,164,326), and hydrogenation generates "hardening" flavors that are removed by further refining. The latter arise from small amounts of free or bound aldehydes, especially 6-trans-nonenal, which can sometimes be detected at a dilution of 0.3 ppb (230,294) (see Description section). Kawada et al. (228) emphasized that the volatile products of hydrogenation differ from those of autoxidation, but no studies on breakdown products of autoxidizing hydrogenated soybean oil were found.

No reports were found to suggest that the digestion, absorption, metabolism, or metabolic effects of hydrogenated soybean oil might differ from those of other dietary fats, or might affect the growth or survival time of man or experimental animals differently from other dietary fats (291,397). Many reports indicated that the distribution of the various FA in body fats tends to reflect that in the diet, taking into account the fact that some FA are readily metabolized or liable to inhibit the metabolism of other FA. This was shown by Beare (042), Neudorffer and Lea (318), Imaichi et al. (200), and Højlmer and Aaes-Jørgensen (139) using complete fats or oils; and by Aaes-Jørgensen (004) and Mohrhauer and Holman (309,310) using isolated FA as sole or principal sources of fat intakes. Van der Steur (412) pointed

Drug Inter-
actions

Consumer

Exposure

relevance to atherosclerosis does not emerge from data (247,351,384) reviewed in the monograph.

No reports were found on soybean oil and drug interaction.

In 1970, 8261 million pounds of soybean oil were produced in the United States, of which 2182 million pounds went into shortenings (total production 3599 million pounds) and 1410 million pounds into margarine (total production 1794 million pounds). The soybean oil contributions to shortenings and margarine were slightly less in 1971, but in all have approximately quadrupled since 1949 (404). No data are given on hydrogenated soybean oils, but the section on Description indicates that much of this oil is partly hydrogenated.

No data on hydrogenated soybean oils are given in the NAS NRC Comprehensive GRAS Survey (137), and no estimate can be made from the survey findings as to the extent of per capita exposure. Nevertheless the tables referring to "Soybean Oil" are abstracted in this monograph (Table 14 and 15).

CHEMICAL INFORMATION

I. Nomenclature

- A. Common names: Soybean oil (hydrogenated), hydrogenated soybean oil, hydrogenated soya bean oil, partly or partially (sic) hydrogenated soybean or soya bean oil. Derived from the seeds of Glycine max (L.) Merr. (G. soja).
- B. Chemical names: None for the entire product, which is a mixture.
- C. Trade names: Same as common names.

D. Chemical Abstract Services Unique Registration Number:

MX 8016 70 4

NOTE: Because hydrogenation is always incomplete and variable, and the results are hard to define precisely, much of the available research information concerns unhydrogenated soybean and analogous oils. Some of this information is included in this monograph as supporting data.

II. Empirical Formula

The composition of fresh soybean oil varies widely according to geography, soil, climate, and seasonal weather. Data claimed to be typical but not average are given in Table 1, adapted from Altman and Dittmer (022), and in Table 2, adapted from Fineberg and Johanson (134). Different data are shown in Table 3, adapted from Erlandsen (125).

The fatty acids (FA) are present mostly as triglycerides, with a small but uncertain fraction as free fatty acids (FFA). About one-half of the FA are polyunsaturated fatty acids (PUFA), and about 90% of the PUFA have two double bonds. The unsaponifiable fraction contains mainly sterols, including cholesterol.

Hydrogenation results in chemical changes that are even more variable. According to Ackman et al. (013), "The partial hydrogenation of fats and oils to produce a stable, palatable product of suitable plasticity results in the formation of new, structurally altered fatty acids which in most cases differ chemically from familiar natural products." Table 4 shows the variable effects of hydrogenation reported from four different laboratories. The FA composition of a partly hydrogenated mixture of vegetable oils that included soybean oil has been described by Kuemmel and Chapman (248).

Composition in terms of glycerides is altered accordingly (see Table 4, upper left) but is rarely reported. Handschumaker et al. (166) observed dipalmito and distearo glycerides after hydrogenation of a mixture that included soybean oil.

Table 1
Typical Composition of Fresh Soybean Oil*

Concentration g/100 g total fatty acids	Identity of Fatty Acid Components			Common Name of Components	
	FASEB Serial # Table 42	Name	Empirical formula	FASEB Serial # Table 43	Name
<u>Saturated Fatty Acids</u>					
0.2	(12)	Dodecanoic acid	$C_{12}H_{24}O_2$	(257)	Lauric acid
0.1	(14)	Tetradecanoic acid	$C_{14}H_{28}O_2$	(258)	Myristic acid
9.8	(16)	Hexadecanoic acid	$C_{16}H_{32}O_2$	(259)	Palmitic acid
2.4	(18)	Octadecanoic acid	$C_{18}H_{36}O_2$	(260)	Stearic acid
0.9	(20)	Eicosanoic acid	$C_{20}H_{40}O_2$	(261)	Arachidic acid
<u>Unsaturated Fatty Acids - Monoethenoic</u>					
0.1	(87)	Tetradecenoic (Tsuazuic) acid	$C_{14}H_{26}O_2$	(262)	Tetradecenoic acid
0.4	(95)	<u>cis</u> -9-Hexadecenoic acid	$C_{16}H_{30}O_2$	(263)	Palmitoleic acid
28.9	(117)	<u>cis</u> -12-Octadecenoic acid	$C_{18}H_{34}O_2$	(264)	Oleic acid
<u>Unsaturated Fatty Acids - Dienoic</u>					
50.7	(159)	<u>cis</u> -9, <u>cis</u> -12-Octa- decadienoic acid	$C_{18}H_{32}O_2$	(265)	Linoleic acid
<u>Unsaturated Fatty Acids - Trienoic</u>					
6.5	(180)	<u>cis</u> -6, <u>cis</u> -9, <u>cis</u> -15- Octadecatrienoic acid (γ)	$C_{18}H_{30}O_2$	(266)	Linolenic acid
	(191)**	<u>cis</u> -9, <u>cis</u> -12, <u>cis</u> -15- Octadecatrienoic acid (α)	$C_{18}H_{30}O_2$		
0.27%				(267)	Sterols

* Abstracted from K.S. Markley (Table 42) and R.P. Geyer (Table 43) in Altman and Dittmer (022).

** Linolenic acid is stated to have two isomeric forms (α and γ) with indistinguishable properties.

Table 2

Some Constants of Fresh Soybean Oil*

1. Extent of unsaturation (percent composition):	
Saturated	12.3 - 15
Monoenes	18.2 - 35
Dienes	44.5 - 57.2
Trienes	5.0 - 9
2. Acid chain lengths (percent composition):	
C ₁₂	0.2
C ₁₄	0 - 0.2
C ₁₆	8.0 - 14.9
C ₁₈	89 - 92
C ₂₀	0 - 1
3. Iodine number	120 - 141
4. Saponification value	189 - 195
5. Unsaponifiables, percent	1.5 maximum
6. Free fatty acid content	"Reliable data unavailable"

* Adapted from Fineberg and Johansen (134)

Table 3

Another Typical Composition of Fresh Soybean Oil*

<u>Fatty acid</u>	<u>Percent</u>
Myristic	0.6
Palmitic	7.0
Stearic	5.8
Oleic	60.6
Linoleic	20.8
Linolenic	5.2
<hr/>	
<u>Constant</u>	<u>Value</u>
Iodine number	129-137
Saponification value	192
<hr/>	

* Adapted from Erlandsen (125)

Table 4

Compositional Changes in Oils by Hydrogenation

A. Gill and Yu (144) - Soybean oil

		Saturated Glycerides x	Per Cent		Fat A	Iodine Number Liquid Acids B
			Olein y	Linolin z		
Run 6	(1).....	28.5	53.5	18.0	78.2	114.1
	(2).....	34.2	53.7	12.1	66.6	100.0
	(3).....	43.4	54.5	2.1	50.2	92.7
Run 7	(1).....	16.5	70.0	13.5	82.1	103.5
	(2).....	39.0	56.2	4.8	56.6	97.3
	(3).....	46.3	53.7	0.0	43.9	86.2
	(4).....	47.5	52.5	0.0	40.5	80.3

B. DeJonge et al. (113) - Soybean oilAnalytical Results of Soya-bean Oil Hydrogenated with a Nickel
(Nos. 1-5) or a Copper (Nos. 6-9) Catalyst

No.	Iodine value	Composition* (wt. %)					Trans fatty acid con- tent (wt.%)	S ₁
		C 18:3	C 18:2	C 18:1	C 18:0	C 16:0		
0†	132.3	8.0	52.0	24.0	4.0	11.5	0	--
1	120.0	4.5	44.5	35.0	5.5	12.0	6.5	2.5
2	111.4	4.0	37.5	39.5	5.0	12.0	12.0	2.0
3	102.7	3.0	32.0	45.0	6.0	12.0	19.5	2.0
4	98.5	2.0	28.0	52.5	6.0	11.0	23.0	2.0
5	91.7	0.7	23.5	68.0	7.0	11.0	25.5	2.5
6	127.0	5.5	53.0	26.0	4.0	11.5	4.0	6.0
7	120.8	2.7	51.5	31.5	3.5	11.0	7.0	8.3
8	115.3	1.5	46.5	35.0	4.0	11.0	9.0	7.0
9	112.1	0.7	41.5	40.0	4.0	11.0	13.0	8.0

* C 18:3 linolenic acid, C 18:2 linoleic acid, C 18:1 oleic acid, C 18:0 stearic acid, C 16:0 palmitic acid, as determined by gas liquid chromatography.

† Original oil.

Fatty Acid Composition and Amount of Natural Linoleic Acid in
Soya-bean Oil Hydrogenated with a Copper Catalyst

Iodine value	Composition (wt. %) from gas liquid chromatography					Content of natural linoleic acid (wt. %)	% Natural linoleic acid of gas liquid chromatography
	C 18:3	C 18:2	C 18:1	C 18:0	C 16:0		
183.5*	7.5	51.0	25.5	3.5	11.5	50.7	100
111.3	Trace	44.5	39.5	4.0	11.0	34.4	77
112.8	Trace	46.0	38.5	4.0	11.0	39.9	87
114.2	0.8	46.5	37.0	4.0	10.5	38.6	83
119.2	2.0	50.0	32.5	3.5	11.5	36.4	73

* Original oil.

Table (Cont.)

Compositional Changes in Oils by Hydrogenation

C. Popescu et al. (341) - Soybean oil

Analytical Results of Hydrogenated Soybean Oil

Experiment no.	% Fatty ester GLC				Iodine value calc.	Alkali isomerization			
	St ^a	M ^a	D ^a	T ^a		% Lo ^a	% Le ^a	% Conj. dienes	% trans
Original soybean oil for Exp. 1 and 2	4	25	53	10	136	50.2	8.3
Original soybean oil for Exps. 3 to 7	4	25	52	9	136	45.5	7.5
1	4	48	38	1	108	28.1	0.0	0.6	18.9
2	4	61	24	0	94	10.0	0.0	0.0	29.6
3	5	63	23	0	94	23.1	2.2	0.0	32.3
4	5	48	36	3	110	5.6	0.0	0.0	21.7
5	5	72	14	0	83	11.1	0.2	0.0	31.6
6	5	66	20	0	91	68	0.0	0.0	27.1
7	16	73	2	0	68	28.0	0.0	0.0	42.6
	4	51	36	0	106				19.5

^a St, stearate; M, monoenoate; D, dienoate; T, trienoate; Lo, linoleate; Le, linolenate.

D. Lambertsen et al. (250) - Fish oilRelative Peak Areas From GLC of Diacetates From Monoenoic Acids in Hydrogenated Fish Oil Cleaved by Reductive Ozonolysis^a

Chain length of monoenoic acid	Geometric isomer	Chain length of dialcohol									
		7	8	9	10	11	12	13	14	15	16
16	cis	15	60	100	80	52	27	16	10		
	trans	10	56	100	90	54	38	21	12		
18	cis	18	60	100	94	80	55	36	25		
	trans	12	67	100	98	88	73	45	—		
20	cis	14	37	61	78	100	71	42	25	13	8
	trans	8	18	46	73	100	73	47	29	20	13
22	cis		20	49	77	100	67	41	20	13	9
	trans		12	30	68	100	76	43	24	16	12

^aMaximal peak area, 100.

The chemical aim of hydrogenation is to saturate selectively double bonds on polyene chains but not on monoene. Achieving this result is difficult; two other effects of hydrogenation are migration of double bonds and cis-trans isomerization (Hannewijk 167). These effects have been studied in monoene FA prepared from fish oils by Ackman et al. (013) and by Lambertsen et al. (250).

Tsuchiya and Akiyama (402) heated hardened soybean oil at 270-280°C for one to two hours with glycerol and reported a "marked degree" of re-esterification.

Hydrogenation gives rise to volatile by-products. Silveira et al. (370) hydrogenated a soybean oil of peroxide value 11.2 meq/kg with Ni catalyst 0.125% at 180°C, and fractionated an ether solution of the volatiles by gas chromatography (GC); fractions were further identified by infra-red and mass spectra. Twenty-one of 41 GC peaks were collected and 10 were identified (Table 5, A). The authors concluded that most of the volatile by-products were hydrocarbons but could only guess at their sources in the fresh oil.

Kawada et al. (228) hydrogenated a soybean oil of peroxide value 10.5 meq/kg at 120°C and identified 37 of 45 GC peaks from a CCl₄ solution of the volatiles (Table 5, B). Four of five unsaturated hydrocarbons were trans isomers. Peroxide value was little changed by hydrogenation. The authors concluded that refinement and deodorization of the oil immediately before hydrogenation could reduce the volatiles by 30-fold (confirmed by Hannewijk, 167), and pointed out that most commercially hydrogenated oils had peroxide values of only 0.5-2.0 meq/kg.

Table 5

Volatile By-Products of Hydrogenation of Oils

A. Soybean oil (Silveira et al. 370)

Gas Chromatographic Fractions Identified

Fraction number	Identified as
1	Acetone (tentative)
2	n-octane
3	n-nonane
5	n-hexanal
6	n-decane
10	n-hexanol
12	3-nonanone (by mass spectrum)
13	n-heptanol
15	n-decanal
18	n-decanol
20	n-heptadecane (by mass spectrum)

B. Soybean oil (Kawada et al. 228)Compounds Identified as Volatile By-Products
of Catalytic Hydrogenation

Peak No. in gas chromatograms	Size of peak	Identified as
1. Saturated hydrocarbons		
3-A	Small	n-Hexane
7-A	Medium	n-Octane
13-A	Medium	n-Nonane
19-A	Medium	n-Decane
24-B	Small	n-Undecane
29-A	Small	N-Dodecane
40-A	Medium	n-Tetradecane
42-A	Small	n-Hexadecane
44-A	Medium	n-Heptadecane

Table 5 (Cont.)

Peak No. in gas chromatograms	Size of peak	Identified as
2. Unsaturated Hydrocarbons		
8-A	Small	<i>trans</i> -Octene
10-A	Small	<i>cis</i> -2-Octene
14-A	Small	<i>trans</i> -Nonene
20-A	Small	<i>trans</i> -Decene
31-C	Small	<i>trans</i> -Dodecene
3. Saturated alcohols		
12-A	Small	n-Propanol
18-A	Small	n-Butanol
23-A	Small	n-Pentanol
28-A	Large	n-Hexanol
33-A	Large	n-Heptanol
36-A	Medium	n-Octanol
39-A	Medium	n-Nonanol
41-A	Large	n-Decanol
4. Esters		
6-A	Medium	Ethyl acetate
5. Carbonyl compounds		
11-A	Small	n-Butanal
17-A	Small	n-Hexanal
34-A	Small	n-Nonanal
38-A	Small	n-Decanal
22-A	Medium	2-Heptanone
27-A	Small	2-Octanone
32-A	Small	2-Nonanone
15-A	Small	3-Hexanone
26-A	Medium	3-Octanone
31-B	Medium	3-Nonanone
25-A	Small	4-Octanone
30-C	Small	4-Nonanone
6. Lactones		
37-A	Small	γ -Hexalactone
45-A	Small	γ -Nonalactone

Numerals indicate the number of gas chromatographic peaks with Ucon Polar as stationary phase (same as the numbers in Fig. 1).

Letters indicate the number of gas chromatographic peak when re-chromatographed with SE-30 as stationary phase.

In short, the composition of fresh soybean oil varies. Hydrogenation is never complete; the degree differs from maker to maker; and the results vary. The composition of partly hydrogenated soybean oil is extremely variable and has never been fully determined.

III. Structural Formula

It follows from the preceding section that no meaningful structural formula can be given for soybean oil.

IV. Molecular Weight

Because soybean oil is a mixture, no molecular weight can be stated. Data for some FA components of fresh soybean oil are given in Table 1.

V. Specifications

No mention of hydrogenated soybean oil was found in the Food Chemicals Codex, 2nd Edition, 1972 (088); the National Formulary, 13th Edition, 1970 (026); the U.S. Pharmacopoeia, 18th Edition, 1960 (405); or Synthetic Organic Chemicals, U.S. Production and Sales (U.S. Tariff Commission) (408). No mention of soybean oil in any form was found in these compendia.

International specifications for "edible soya bean oil" have been recommended by the Codex Alimentarius Commission (CAC) of the Joint FAO/WHO* Food Standards Programme (085). Some of these recommendations are abstracted in Table 6. Item 6.1.2 in Table 6 covers hydrogenated soybean oil and implies that it may be different (it is a product, whereas unhydrogenated soybean oil may be classed

* Food and Agricultural Organization and World Health Organization of the United Nations Organization.

as produce). The hydrogenated oil appears to be covered by the FAO/WHO Recommended General Standard for Edible Fats and Oils Not Covered by Individual Codex Standards (083); some of the recommendations are abstracted in Table 7.

In short, no binding specifications that unequivocally describe hydrogenated soybean oil (or its synonyms; see Nomenclature) have been found. Even the CAC recommended standards are not yet in force; they await ratification and adoption by the Member Nations of FAO/WHO.

VI. Description

Although the chemistry of hydrogenated oils is somewhat uncertain, their physical handling characteristics are more predictable; oils are partly hydrogenated in order to elaborate products of improved stability and suitable plasticity, which varies according to the procedures used.

Hannewijk (167) has reviewed the principles of hydrogenation and has emphasized the following points: Crude oils with over 0.1% of FFA must be bleached and neutralized before being hydrogenated, but other impurities including bound aldehydes (which give bad flavor) are removed by normal refining. The hydrogen must be free from S and CO. High temperatures improve the selective hydrogenation of higher PUFA; however, if the oil becomes hotter than about 200°C aromatic FA bound to glycerol tend to be formed. The catalyst usually is solid, of known pore size, and contains Ni. Its contamination with S enhances cis-trans isomerization resulting in a fat that is firmer

Table 6

Codex Alimentarius Recommended International Standard
for Edible Soya Bean Oil (085). Some Abstracted Specifications

2.1.1.	Relative density (20°C/water at 20°C)	0.919 - 0.925
2.1.2.	Refractive index ($n_D^{40^\circ\text{C}}$)	1.466 - 1.470
2.1.3.	Saponification value (mg KOH/g oil)	189 - 195
2.1.4.	Iodine value (Wijs)	120 - 143
2.1.5.	Unsaponifiable matter	not over 15 g/kg
2.2.3.	Acid value	not over 0.6 mg KOH/g oil
2.2.4.	Peroxide value	not over 10 meq peroxide oxygen/kg oil
3.3	<u>Antioxidants and synergists:</u>	
3.3.1.	Gallates, i/c*	100 mg/kg
3.3.2-3.7	BHT, BHA, i/c, with/without gallates	200 mg/kg
3.3.4-5.	Ascorbyl palmitate/stearate i/c	200 mg/kg
3.3.7.	Dilauryl thiodipropionate	200 mg/kg
3.3.6.	Tocopherols	unlimited
3.4.3-5.	Isopropyl citrate mix, monoglyceride citrate, phosphoric acid, i/c	100 mg/kg
3.5	Dimethyl silicon, SiO ₂ , i/c	10 mg/kg
3.6	Oxystearin	1.25 g/kg
4.1	Matter volatile at 105°C	0.2% m/m
4.2	Insoluble impurities	0.05% m/m
4.3	Soap	0.005% m/m
4.4	Iron	1.5 mg/kg
4.5-4.7.	Copper, Lead, Arsenic - each	0.1 mg/kg
6.1.2.	Where <u>soya bean oil</u> has been subjected to any process of esterification or to processing which alters its fatty acid composition or its consistency the name <u>soya bean oil</u> or any synonym shall not be used unless qualified to indicate the nature of the process.	
6.2.1.	A complete list of ingredients shall be declared on the label in descending order of proportion.	

* i/c = individually or in combination

Table 7

Codex Alimentarius Recommended International General Standard
for Edible Fats and Oils (085): Some Extracted Specifications

-
- 3.3 Odor and Taste: free from foreign and rancid odor and taste
- 3.4 Acid Value: Non-virgin fats and oils - not more than 0.6 mg KOH/g fat or oil
- 3.5 Peroxide Value: Not more than 10 meq of peroxide oxygen/kg fat or oil
- 4.3.2-11 Emulsifiers: maximum 20 g/kg inclusive, of a list of 10 classes of substances, individually or in combination
- 4.4-7 Antioxidants, Antioxidant Synergists, Anti-foaming Agents, and Crystallization Inhibitors: A long list of individual maxima
5. Contaminants, Maximal levels:
- | | |
|-----------------------------------|------------|
| 5.1 Matter volatile at 105°C - | 0.2% m/m |
| 5.2 Insoluble impurities - | 0.05% m/m |
| 5.3 Soap - | 0.005% m/m |
| 5.4 Iron (Fe), non-virgin oil - | 1.5 mg/kg |
| 5.5 Copper (Cu), non-virgin oil - | 0.1 mg/kg |
| 5.6 Lead (Pb) - | 0.1 mg/kg |
| 5.7 Arsenic (As) - | 0.1 mg/kg |
7. Labeling provisions include "a complete list of ingredients" (7.2.1); "net contents" (7.3); "name and address" of supplier (7.4).
-

at room temperatures; S contamination accumulates, however, and has to be watched.

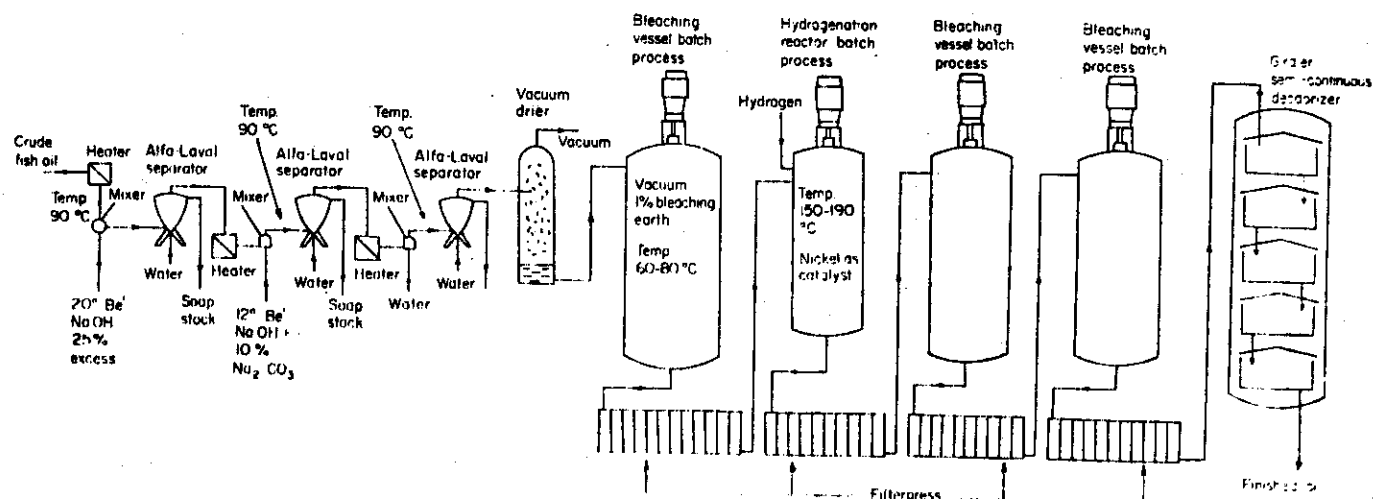
Chang (081) has reviewed the processes in detail, with flow charts (Figure 1) and diagrams of types of apparatus commonly used, including alternatives. He comments that while Cu-containing catalysts hydrogenate more selectively than Ni catalysts (they leave out the monoene FA), they cost more and the products are more prone to oxidize. He notes that oils are finally deodorized at 190-230°C in the United States, but at 170-190°C in Europe to avoid possibly harmful thermal decomposition and polymerization.

Koritala (246) investigated 26 solvents for selective hydrogenation at various temperatures and pressures, and concluded in favor of high polarity, although the chemical mechanisms were unclear. The flavor of soybean oils hydrogenated at lower temperatures was more stable if the process was more selective.

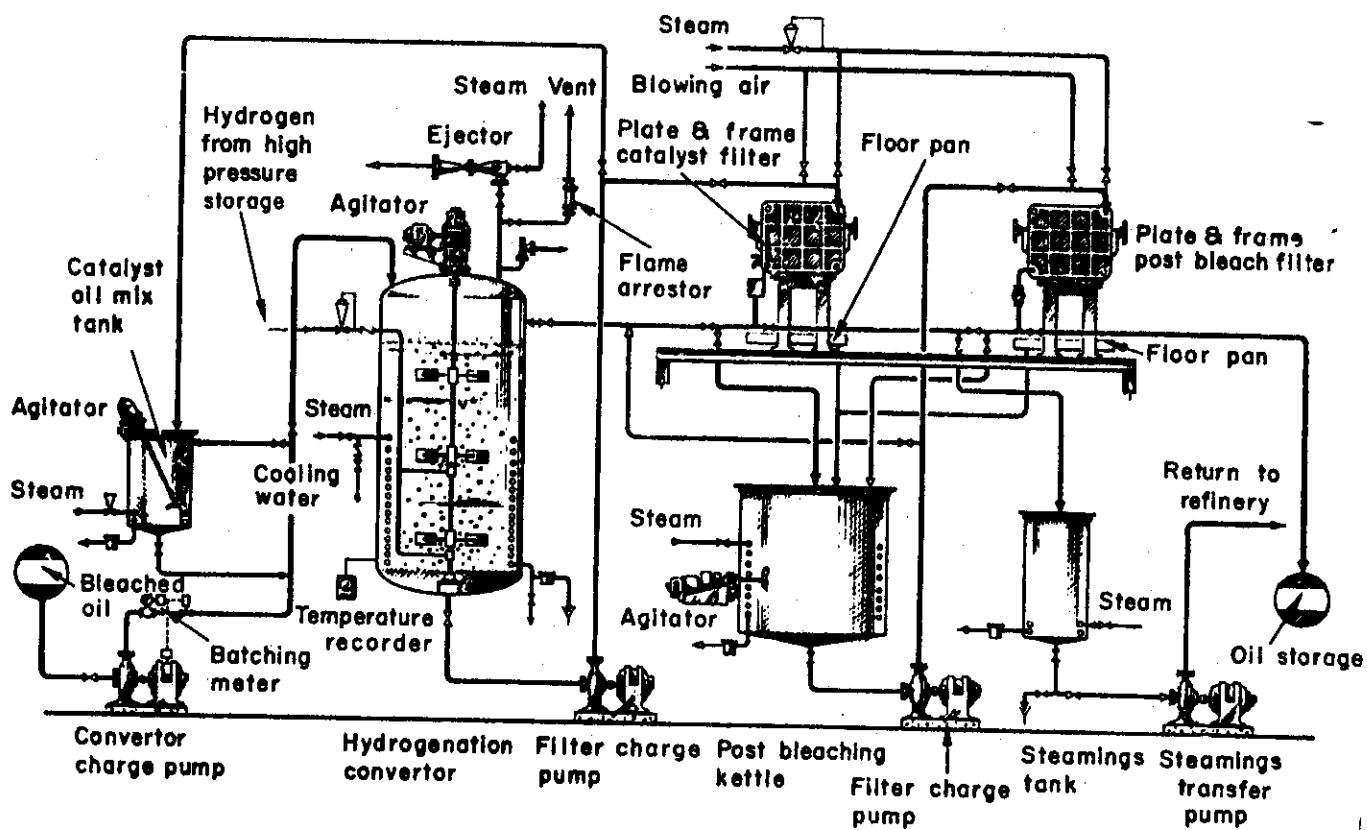
In a continued search for these qualities, Koritala (245) and his coworkers (089) re-examined the merits of Cu-Cr and Cu-oxide catalysts in comparison with Ni. Though the search was inconclusive they found that bad odor and flavor, as judged by a taste panel, was related to levels of linolenate and isomers of linoleate in the finished oils.

Popescu et al. (341) reported production of highly monoene (oleate) soybean oil using a Cu-Cr-Ni mixture as catalyst, and that Cu residues were "readily removed" by refining or treatment with citric or phosphoric acids. Beal et al. (041) compared many methods of removing Cu residues and concluded in favor of treatment with cation-exchange resin followed by deodorization with 0.01% citric acid.

**Fig. 1. General Procedures of Hydrogenation
From Chang (081)**



A. Flow Sheet of Fish-Oil Processing



**B. Lay-out of a Hydrogenation Plant
(From Bailey's Industrial Oil and Fat Products by Daniel Swern.)**

Table 8

Some Physical Characteristics of Soybean Fatty Acids*

FASEB Serial No.	Iodine Number**	Molecular Weight	Melting Point °C	Boiling Point °C (at mm Hg)	Specific Gravity (at °C)	Refractive Index*** (at °C)	Soluble in****
(12)	0	200.3	44.2	225(100)	0.8690(50)	1.4261(60)	acet,alc,eth,pet.eth.
(14)	0	228.4	53.9	250(100)	0.8622(54)	1.4273(70)	acet,alc,eth,pet.eth.
(16)	0	256.4	63.1	268(100)	0.8487(70)	1.4309(70)	acet,h.alc,eth,pet.eth.
(18)	0	284.5	69.6	213(5)	0.8390(80)	1.4337(70)	acet,h.alc,eth,pet.eth.
(20)	0	312.5	76.5-77.0	201(1)	0.8240(100)	1.4250(100)	bz,chl,eth,pet.eth.
(87)	112.13	226.35	18-18.5	185(13)	0.9024(20)	1.4559(20)	bz,pet.eth.
(95)	99.77	254.40	-0.5 to +0.5	140-141(5)	-	-	-
(117)	89.86	282.45	9.8-10.4; 26.5-27.5	196(1.5)	-	-	-
(159)	181.01	280.44	-5	229-230(16)	0.9031(20)	1.4711(20)	acet,alc,eth,pet.eth.
(180)	273.51	278.40	-11.3 to -11	125(0.05)	0.9164(20)	1.4800(20)	acet,eth,me.alc,pet.eth.
(191)	273.51	278.40	-11.3 to -11	125(0.05)	0.9164(20)	1.4678(50)	acet,alc,eth,pet.eth.

* Identified in Table 1, compare FASEB serial numbers. Not hydrogenated.

** Grams of I absorbed by 100 g of acid. Sp. Gr. referred to water at 4°C.

*** At the D-line of Na (589nm).

**** acet = acetone; alc = alcohol (ethyl unless otherwise specified); h = hot; me = methyl;
eth = diethyl ether; pet = petroleum; bz = benzene; chl = chloroform.

Gaps mean no data reported in Table 2 ().

Miyake et al. (305) have attributed these and other problems of hydrogenation to incompatible kinetics of the various reactions.

In short, the catalyst that will hydrogenate perfectly and leave no toxic traces has not yet been found. This is evidenced by the continual patenting of claims for minor improvements of method (046,299,314,371) and see General Bibliography).

Hydrogenated oils and fats are mixtures of lipids with very different mp and expansion spectra, which causes them to be described as relatively "steep" or "flat." To some extent these variations are carried over from the starting material, shown in Table 8.

Partly hydrogenated fats and oils are treated with weak alkali to remove traces of FFA and Ni-catalyst and are then bleached and deodorized (see Fig. 1). The question of stability will be considered later (see Biochemical Aspects - Breakdown).

Vitamin losses during refinement, hydrogenation, and finishing have been studied by Loury (254). He found about 20% reduction of the tocopherol levels present in crude soybean oil. Although the antioxidant properties of tocopherols are important for keeping quality (see Breakdown), Loury did not consider the losses to be significant.

Hydrogenation results in some aversive "hardening flavors." Hannewijk (167) states that they vary with the degree to which the PUFA are saturated and with the mp spectrum, being greater at lower mp. He attributes these results to the presence of free ketones and especially free aldehydes, and notes that levels above taste thresholds can develop within hours in daylight and about 2 months

in the dark.

Meijboom (294) tried to relate the odors and tastes of some pure saturated and unsaturated aldehydes to their molecular structures. He found, as have others, that the technical problems are enormous. Today these problems remain unsolved (077). Nevertheless, Meijboom and his coworkers (230) appear to have identified the principal carrier of hardening flavor in hydrogenated linseed and soybean oils as 6-trans-nonenal, which was detected by some subjects at a dilution of 0.3 ppb.

In short, partly hydrogenated soybean oil has no precise physical description. Subjective, empirical judgments, based on reports by sensory evaluation panels, tend to govern the commercial acceptability of the diverse forms of such oil.

VII. Analytical Methods

Probably no complete analysis of any sample of partly hydrogenated soybean oil has ever been made.

No records were found of officially specified methods. Recommended methods for estimating compliance with the CAC Recommended International Standards await ratification. These are listed in the Standards for Edible Soybean Oil (085) and Edible Fats and Oils (083) (see Specifications); they also appear in the Standard for Margarine (084) to the extent that this product contains a high ratio of hydrogenated soybean oil (see Consumer Exposure).

O'Connor et al. (322) reported a method for detecting soybean oil when it is mixed with cottonseed and peanut oils, which do not contain linolenic acid. The approximate number of triene conjugations

is determined by spectrophotometry at 268 nanometers; this, however, is not quantitative for soybean oil (see Empirical Formula) and is useless after part-hydrogenation of the oils.

Ueno (409) found a relationship between iodine number and thiocyanogen value determined by "the usual method, employing the pure acetic acid," which, he claimed, reflected the degree of hydrogenation of a fish oil.

Feuger and Bailey (131) described a physical estimate of hydrogenation, using a "micropenetrometer" with a sharp needle. They plotted temperature against "micropenetrations" of 0-25 mm, drew regular curves for iodine number sequences in partly hydrogenated peanut, cottonseed, and soybean oils, and claimed that the results indicated degrees of plasticity.

Malins and Mangold (266) and Mangold (272) reported that thin layer chromatography (TLC) could be used to analyze complex lipid mixtures, using equipment and techniques that are now standard. Mangold (272) claimed advantages over previously existing methods in simplicity, speed, efficiency, sensitivity, and capacity, and he recognized that the chief use for TLC in lipid analysis would be for preparative fractionation. His paper contains 235 literature references and is cited as authoritative in many recent reports included in this monograph.

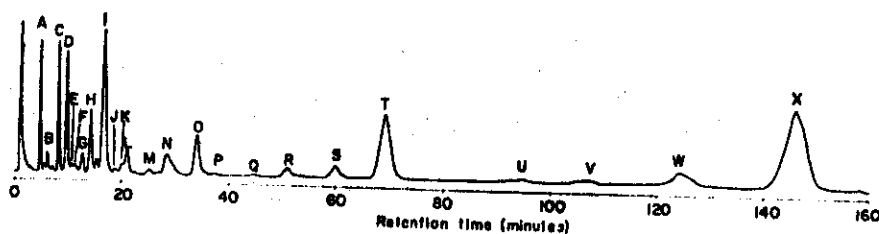
The state of the art in 1967 was reviewed by Malins and Wekell (267). Mangold and his coworkers (221) have continued to extend their TLC techniques, concentrating on adsorbents with special affinities for separating the more recondite fractions of natural

oils, including soybean oils.

The FA spectrum in natural oils, including partly hydrogenated soybean oil, is now generally determined by gas or gas-liquid chromatography (GLC) of TLC fractions. This work has been reported by many authors including Karmen *et al.* (218) and Kuemmel and Chapman (248). Principles, techniques, analytical standards, and some results, are summarized by Houle (197), some of whose illustrations are reproduced in Figure 2.

Fig. 2. Examples of Gas-Liquid Chromatographic Analysis of Natural Oils for Fatty Acid Spectrum

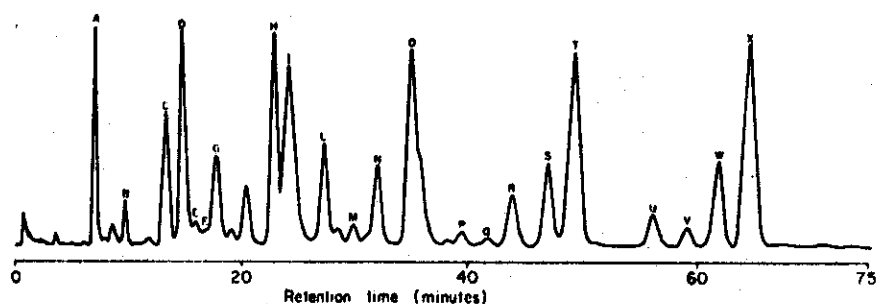
From Houle (197)



A. Isothermal GLC Analysis of the Fatty Acid Methyl Esters of Menhaden Oil

Instrument: Aerograph 6000, hydrogen flame ionization detector.
Column: Ten percent diethyleneglycol succinate on 100/120 mesh Chromosorb W. Ten feet times one-eighth inch outside diameter pyrex glass column. Conditions of analysis: Temperature, 178°C; Nitrogen carrier gas flow rate, 30 ml/min.; Hydrogen flow rate, 24 ml/min.; and Air flow rate, 400 ml/min. Identification of individual peaks on the chromatogram. (Fatty acid chain length; number of double bonds). A, 14:0; B, 15:0; C, 16:0; D, 16:1; E, 17:0; F, 16:2; G, 17:1; H, 18:0; I, 18:1; J, 19:0; K, 17:3 or 18:2; L, 18:2; M, 20:0; N, 18:3; O, 20:1; P, 20:2; Q, 22:0; R, 22:1 or 20:4, S, 20:4; T, 20:5; U, 24:1 and 22:4; V, 22:4; W, 22:5; and X, 22:6.

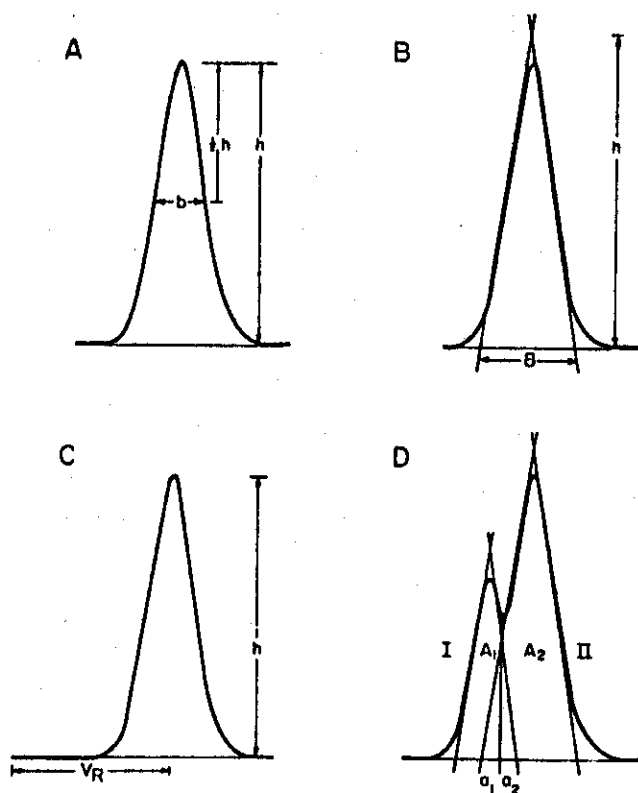
Fig. 2 (cont.)



B. Programmed Temperature GLC Analysis of the Fatty Acid Methyl Esters of Menhaden Oil

Instrument: Barber-Coleman Model 5000, dual hydrogen flame ionization detectors. Column: Six feet times one-quarter inch outside diameter, packed with five percent stabilized diethyleneglycol succinate on 80/90 mesh Anakrom ABS. Conditions of analysis: Column temperature at start of run, 130°C. Temperature program rate of 1°C/min. Nitrogen carrier gas flow rate of 60 ml/min.; Hydrogen flow rate of 39 ml/min.; Air flow rate of 400 ml/min. Identification of individual peaks on the chromatogram. (Fatty acid chain length; number of double bonds). A, 14:0; B, 15:0; C, 16:0; D, 16:1; E,F,G, identity uncertain; H, 18:0; I, 18:0; L, 18:2; M, 20:0; N, 18:3; O, 20:1; P,Q, identity uncertain; R, 22:1 or 20:4; S, 20:4; T, 20:5; U, 24:1 and 22:4; V, 22:4; W, 22:5; and X, 22:6.

Fig. 2 (cont.)



C. Common Methods of Measurement of Peak Areas

A, Peak height times base at one-half height. B, Triangulation; Peak height times one-half the base. C, Retention time times peak height. D, Measurement of incompletely resolved peaks by triangulation.

Attempts to measure "plasticity" using objective, modern technology have been reported. For example, Pohle and Gregory (337) described a stage in the development of standards for determination of solids in fats and shortenings by nuclear magnetic resonance, and concluded that confirmation of their results by others was required.

The phospholipid fraction of soybean oil is about one-tenth the amount of the triglyceride fraction and can be measured by nitric-perchloric determinations of lipid phosphorus (for which the laboratory should be equipped with shatter-proof glass). Zhukov and Vereshchagin (429) have proposed some modifications that would reduce sample volume and improve both accuracy and safety.

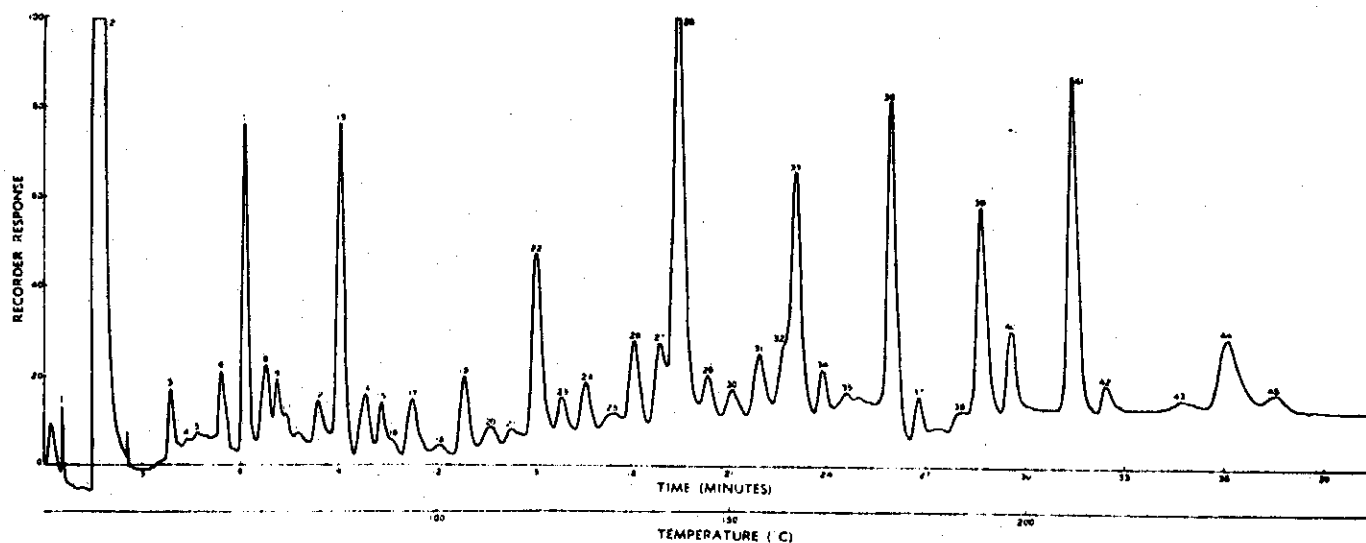
Ohlson (323) determined oxygen sorption in a number of oils including soybean oil under various conditions. He used a pH meter with an O electrode that measured a unit proportional to the partial pressure of oxygen. He found that hydrogenated oils absorbed oxygen more rapidly than raw oils.

Determination of oxidative rancidity using 2-thiobarbituric acid reagent was reported for fish oils by DeKoning and Silk (114). This is a standard method that determines the amount of malonaldehyde arising from oxidative decomposition of polyene PUFA. Results were found to be parallel to peroxide values.

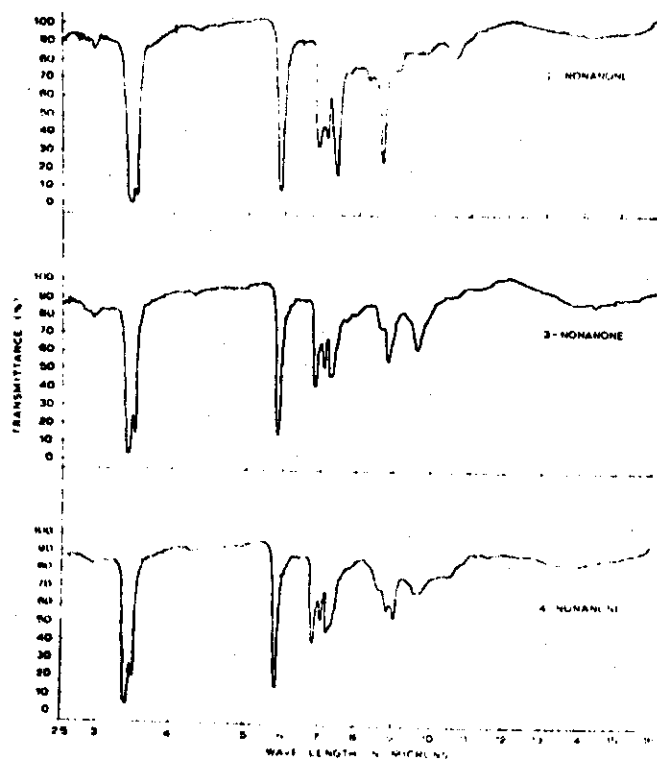
Chang and his coworkers (370,228) described the identification of volatile by-products of hydrogenation of soybean oil (see Table 5), principally by gas chromatography. Figure 3 illustrates some typical data.

Fig. 3. Gas Chromatogram of Volatiles of Hydrogenated Soybean Oil
Studied Further by Infrared and Mass Spectra

From Kawada *et al.* (228)

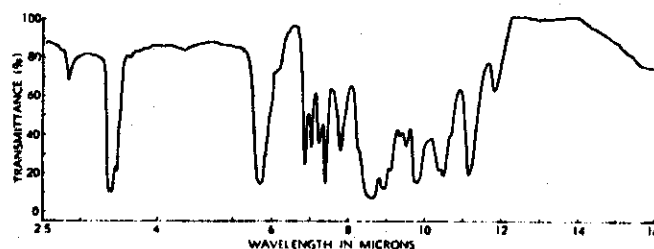


A. Gas chromatogram of the volatile by-products developed during
hydrogenation of soybean oil

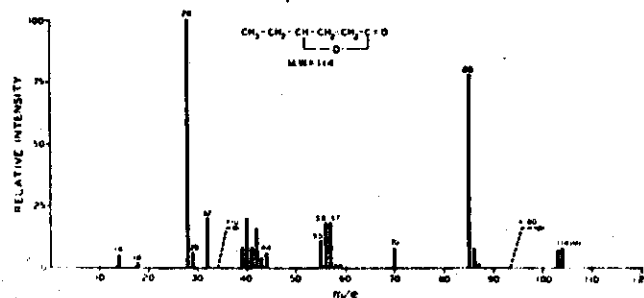


B. Infrared spectra of 2-nonanone, 3-nonanone, and
4-nonanone

Fig. 3 (cont.)



C. Infrared spectrum of the fraction 45-A identified as γ -hexalactone



D. Mass spectrum of the fraction 45-A identified as γ -hexalactone

Copper residues in edible oils were determined by Deck and Kaiser (109) colorimetrically in EDTA solutions reacted with a sensitive phenanthroline compound. They claimed determinations of 0.002-0.1 ppm.

Arsenic and bromine determinations in edible oils down to 0.01 ppm were claimed by Lumde (257) after samples were fractionated on silica gel columns and submitted to neutron activation analysis.

VIII. Occurrence and Levels Found in

- A. Plants. In 1970, in the USA, 761,265,000 bushels of soybeans yielded 8,261,000,000 pounds of oil, which is approximately 11 pounds per bushel (Agricultural Statistics 404).
Yields of course vary from year to year and place to place.
- B. Animals. Not applicable.
- C. Synthetics. Not applicable.
- D. Natural Inorganic Sources. Not applicable.

BIOLOGICAL DATA

I. Acute Toxicity

No reports have been found on acute toxicity of soybean oil.

Hydrogenated soybean oil is commonly regarded as a major nutrient, and major nutrients are not commonly regarded as acutely toxic.

Nevertheless, it may be relevant that Matsuo studied acute toxicity in fish oils for many years, and isolated and purified a cyclic monomer that proved acutely toxic to rats and rabbits. He (286) concluded that this monomer is an endproduct of autoxidation and peroxidation of many edible oils. Confirmation has not been found in the literature for hydrogenated soybean oil.

Woods et al. (423) found a potentially lethal dermatitis when chicks were fed 20% of their diet as vegetable oil. To see if this effect was due to skin contamination with diet, the authors applied a number of oils to the skin of 2-week-old chicks; those receiving unhydrogenated soybean oil developed severe skin lesions, and two out of five died within five days. When hydrogenated "vegetable" oil was used, one of six died. All of the oils were judged equally toxic when applied in this way. When the authors found that most of the damage could be prevented by adding antibiotics to the oils, they concluded that secondary infections were responsible.

Hakansson (163) looked for changes in an artificial soybean oil emulsion intended for intravenous injection and found increasing FFA levels at 6-36 months of storage. The aged emulsions were injected into dogs, and cell damage was reported in a number of

internal organs, together with changes in serum lipid and protein values. No reports have been found of similar effects of FFA from hydrogenated soybean oils administered orally.

II. Short Term Studies

Reports directly or indirectly relevant to hydrogenated soybean oil deal with five sorts of effect: (a) direct toxicity of the oil, (b) nutritional imbalance, (c) EFA deficiency, (d) vitamin E or antioxidant deficiency, and (e) autoxidation and peroxidation effects. Most of these reports concern highly artificial experiments in animals.

a. Direct Toxicity

Thomasson et al. (399) fed groups of 24 male and 24 female rats a 60% (by kcal) fat diet for 12 weeks. Of the 60%, 54% was one of the fats shown in Table 9, and 6% was sunflower oil to provide EFA activity. The three soybean oils L5, L15, and L40 were hydrogenated in different ways.

Table 9

Dietary Fat Used in Short Term Toxicity Study
by Thomasson et al. (399)

Percentage FA composition of dietary fats

Type of experimental fat	Fatty acids				
	Saturated	Monoene		Diene	
		<i>cis</i>	<i>trans</i>	<i>cis-cis</i>	<i>trans</i>
Soya-bean oil (control)	15	24	0	55	0
Hydrogenated soya-bean oil (L ₅)	21	12	54	6	4
Hydrogenated soya-bean oil (L ₁₅)	16	47	1	16	19
Hydrogenated soya-bean oil (L ₄₀)	15	22	14	41	7
Hydrogenated linseed oil	16	20	40	6	16
Hydrogenated olive oil	24	39	29	6	1
Butterfat	53	24	8	7	3

No significant differences from controls were found in food intake; body-weight gain; fecal fat; water intake; rectal temperature; kidney function as measured by urine s.g. and aspartate transaminase activity; liver function as measured by alkaline phosphatase and serum alanine transaminase activities; hemoglobin; hematocrit; hemolysis time in a thiourea medium; total and differential leucocyte counts; coagulation; platelet adhesiveness; or (at autopsy) weights and histological appearances of heart, liver, kidneys, adrenals, spleens, or testes. All of these observations appeared normal. Body fat compositions, in terms of saturated FA and of monoenoic, polyenoic, and trans acids, reflected those of the respective dietary fats.

b. Nutritional Imbalance

In a review of the nutritional properties of oils, Stansby (383) contrasted fish and vegetable oils to make the point that EPA should be defined more broadly and PUFA more precisely than many people do. He considered growth support and cholesterol depressant activities to outweigh the curing of dermal symptoms as criteria of EPA. Among PUFA he contrasted the properties of dienes (such as linoleate) that predominate in vegetable oils with those of trienes and higher polyenes that predominate in fish oils. He emphasized that oxidation effects were "all only potential" -- that is, that they reflected deterioration and would not necessarily follow ingestion of oil that was normally fit for human consumption.

Njaa et al. (320) fed young rats a low protein, low choline diet for four weeks. Diet calories were doubled by adding soybean oil hydrogenated to mp 40.5°C, hydrogenated marine fat, hydrogenated

rapeseed oil, natural herring oil, or lard. Herring oil produced some fatty livers. These excluded, the "hydrogenated" groups developed larger livers than the "natural" groups; for cause, the authors ruled out choline deficiency and suggested low linoleate intakes. The "hydrogenated" groups gained less weight, and the authors identified the probable cause as artificial polymerization (during hydrogenation) of C_{20} and C_{22} FA, because the group receiving soybean oil was least affected. Additions of α -tocopherol produced no effect.

Paluszak (333) fed a total of 80 rats one or the other of six diets, which included in some cases a fully (maximally), hydrogenated rapeseed oil. The principal effect of this oil was to eliminate all adipose tissue. Toxic residues in the oil were exonerated, and the author concluded that deficiencies of EFA and biotin were responsible.

c. EFA Deficiency

Aaes-Jørgensen (007) defined substances that would qualify as EFA as follows: "The criteria for a polyenoic acid belonging to the group of essential fatty acids are: a long chain with cis-double bonds in divinyl methane arrangement, and the first double bond in position 6 counted from the methyl end of the molecule (ω -6-position)." Examples given were linoleic acid, ω -linolenic acid, and arachidonic acid, considered as members of the "linoleic acid family." The word "family" reflects convertibility by metabolism in the body. EFA actions included cure of dermal symptoms, restoration of growth, prevention of damage to kidneys and reproductive tissues, and prevention of accumulation of eicosatrienoic acids normally present at

low levels. Unlike Stansby, Aaes-Jørgensen (007) excluded cholesterol-depression as not being "linked exclusively" to EFA. He pointed out that FA of the linolenic (ω -3) family promoted growth but did not cure dermal symptoms, and argued that the oleic-palmitoleic ratio might provide a chemical indicator of EFA deficiency; these could be biosynthesized (by man) while linoleic and linolenic acids could not. Study of these inferences was urged by the author for two practical reasons: the nutritional contribution of PUFA to phospholipids, and their readiness to autoxidize (see below).

Aaes-Jørgensen and Hølmer (008) fed different diets (Table 10) to five groups each of 30 weanling male rats and found that part-hydrogenated soybean oil or natural arachis oil more than doubled the growth rate compared with hydrogenated herring or arachis oils or fat-free diet (Figure 4).

Table 10

Dietary Fats Used in Short Term EFA Study by
Aaes-Jørgensen and Hølmer (189)

Fatty Acids of Dietary Oils (Wt %)				
Fatty acids, per cent of oil	Partially hydrogenated arachis oil (HAO)	Partially hydrogenated soybean oil (HSO)	Arachis oil (AO)	Partially hydrogenated herring oil (HHO)
Saturated	29.3	19.8	19.1	41.9
Monosenoic	67.0 ^a	41.4 ^b	50.9 ^c	44.8 ^d
Polyenoic	3.3	39.0	29.9	13.2
C ₁₈ :2, ω 6				
Linoleic acid	0.8	31.8	28.8	0.6
C ₁₈ :3, ω 3				
Linolenic acid	—	3.4	1.1	—
Total trans acids (as Elaidic acid)	47	10.7	0	22.9

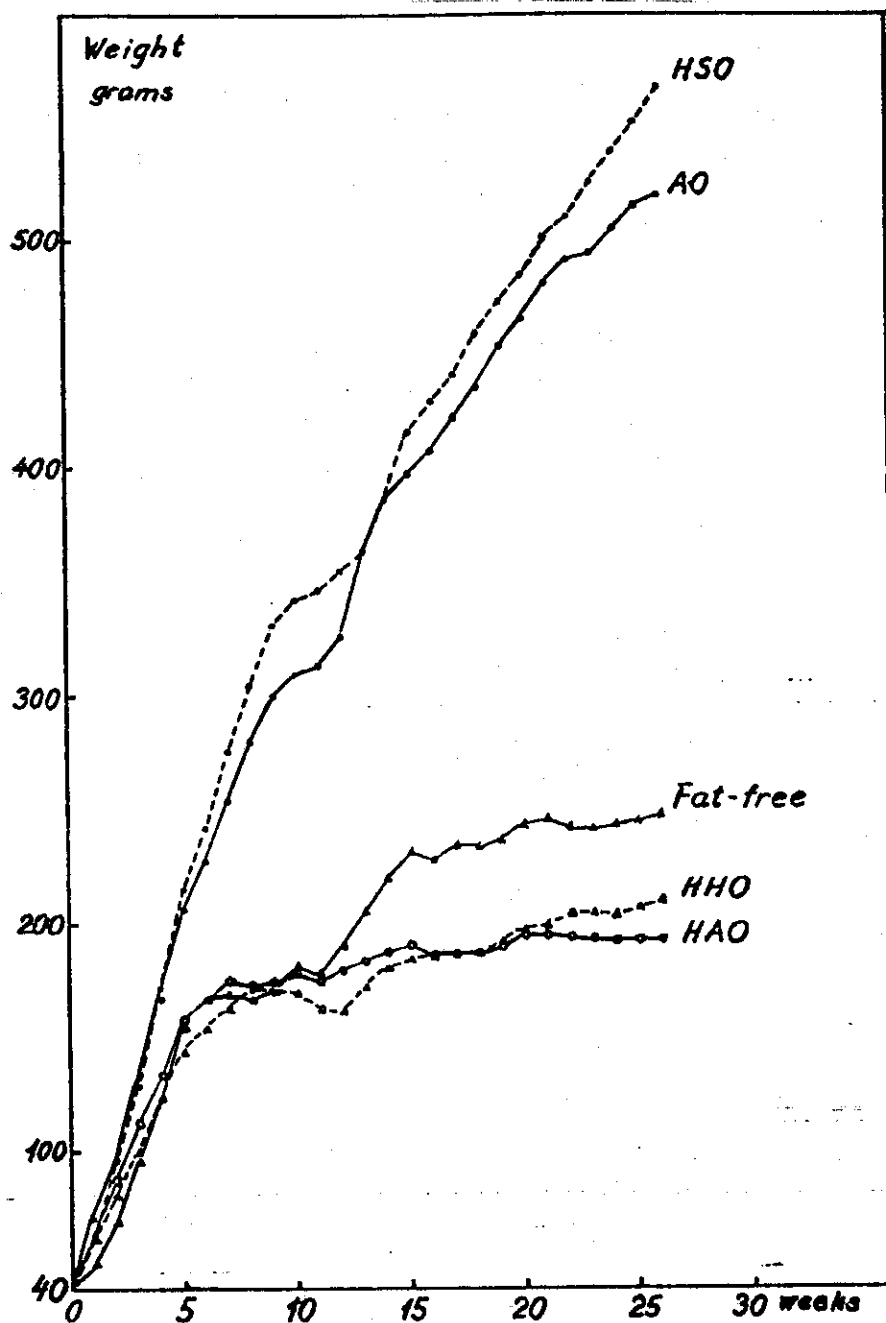
^aC₁₆:1, 1%; C₁₈:1, 64.8%; C₂₀:1, 1.2%.

^bC₁₆:1, 0.6%; C₁₈:1, 40.8%.

^cC₁₆:1, 0.3%; C₁₈:1, 48.5%; C₂₀:1, 1.7%.

^dC₁₆:1, 5.6%; C₁₈:1, 13.0%; C₂₀:1, 11.3%; C₂₂:1, 14.1%.

Fig. 4. Growth Rates of Rats in The Short Term EPA Study by Aaes-Jørgensen and Hølmer (189).



Average growth rate of male rats fed on a diet with 28% partially hydrogenated soybean oil (HSO), 28% partially hydrogenated herring oil (HHO), 28% partially hydrogenated arachis oil (HAO), 28% arachis oil (AO), or a fat-free diet (Fat-free).

The hydrogenated herring oil degenerated the testicles in 5 weeks, the hydrogenated arachis oil in 15, the fat-free diet in 26, and the hydrogenated soybean oil caused no degeneration in 26 weeks. The oil still contained 32% linoleic acid, and a total of 11% of trans acids calculated as elaidic. However, the authors pointed out that the ultimate chemical basis of these different effects remained speculative.

d. Vitamin E or Antioxidant Deficiency

Blaxter et al. (1955) fed 42 neonatal bull calves a ration designed to produce muscular dystrophy; 27 also received either α -tocopheryl acetate, ascorbic acid, biotin, ethyl gallate, or methylene blue. Only α -tocopheryl acetate when given by mouth, and methylene blue, were found to be protective. The authors concluded that antioxidant activity could not be the basis of this protection, and they emphasized that the role of vitamin E was unknown at that time.

Ershoff (1926) fed immature rats a basal low-fat diet containing 71% sucrose and 24% casein supplemented (or not) with one or more of many oils including three levels of soybean oil (2, 5, and 10%). Fish oils resulted in diarrhea and growth retardation; these effects were counteracted by adding 10% soybean, cottonseed, sesame, corn, or wheat germ oils, but not by addition of methyl linoleate, butterfat, lard, or olive, coconut, or hydrogenated cottonseed oils. α -Tocopherol, but not α -tocopheryl acetate, gave some protection, as also did DPPD (N,N'-diphenyl-p-phenylenediamine), Santoquin, sesamol, Torula yeast 10%, desiccated liver NF, alfalfa meal 20%, casein, fishmeal, and a mixture of crystalline amino acids, or DL-methionine 0.6%. The author

concluded that this protection in these animals was due to antioxidant activity.

Dam (099), using chicks, recognized that two separate actions were involved, and suggested that vitamin E was stabilized in the body by other antioxidants, which at the same time duplicated the antioxidant activity of the vitamin.

What should not be forgotten is that basal diets of the type given by Ershoff to young rats cause an immense induction of enzymes of the oxidative and anerobic pathways of glycolysis (360), and that this result greatly influences the metabolism of particular FA (420-- see Biochemical Effects).

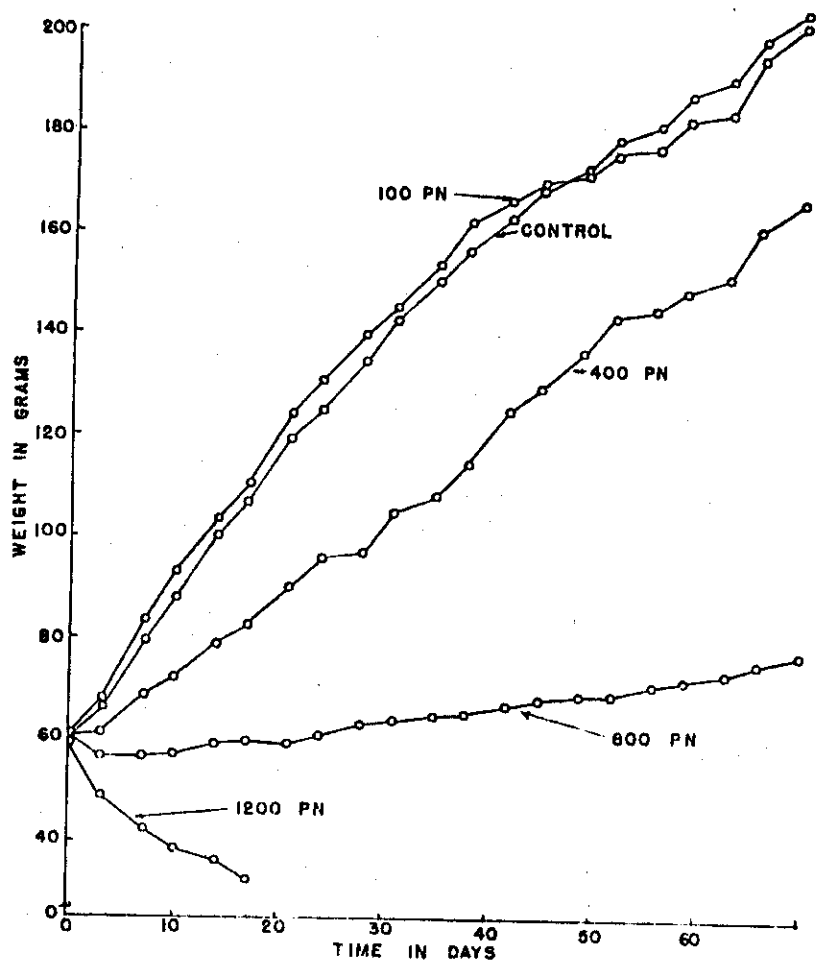
Harris and Embree (169) studied the relationship between intakes of PUFA and requirements for vitamin E in man. They calculated the average intakes of PUFA in g per head of the population of the United States for the year 1960, and the average intakes of Vitamin E expressed as mg of d- α -tocopherol. The ratio, mg of vitamin E to g of PUFA, was found to be 0.6, and the authors inferred from published data that this ratio was probably marginal for protection of man against vitamin E deficiency. However, Scott (367) gives reasons to suspect that the value of this ratio may have been partly due to selenium deficiency. Very recently, the National Academy of Sciences has issued a denial of suggestions that vitamin E deficiency may be widespread in the United States at the present time (317).

e. Autoxidation and Peroxidation Effects

Andrews et al. (029) oxidized some raw soybean oil under controlled conditions to peroxide values of 13, 927, 1156, and 3185,

and found these fractions to contain conjugated dienes at 0, 1190, 780, and 1602 meq/kg respectively (showing further uncontrolled decomposition). They then adjusted the fractions to peroxide values of 100, 400, 800, and 1200, and fed them to four groups of 10 rats each, but not to a fifth group, for 10 weeks as 15% of a diet containing 61% sucrose and 18% casein. The effects on growth of the females are shown in Figure 5.

Fig. 5. Growth of Rats Versus Peroxide Value of Fats
From Andrews et al. (029)



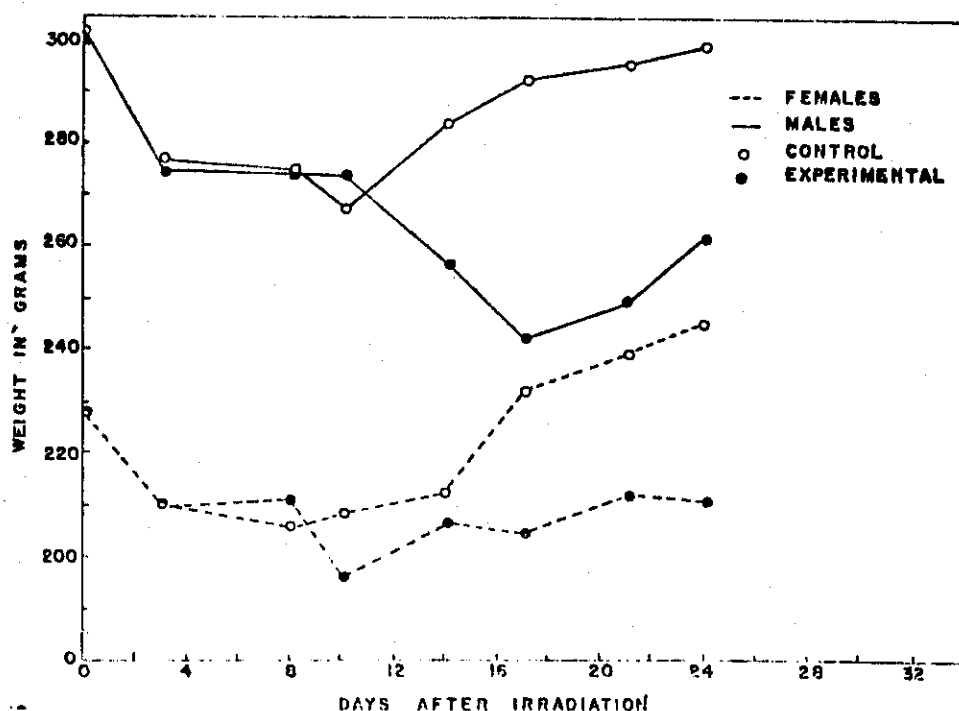
Growth of female rats receiving various levels of oxidized fat

The lowest group all died within three weeks; the authors state that the graph for the males was similar. Although the diet containing oil of peroxide value 100 had no effect on growth in this experiment, it severely diminished growth during recovery from whole-body irradiation (Fig. 6A). These and other studies showed that the growth effect was related quantitatively to the peroxide value. At autopsy it seemed probable that the intestine was the site of toxicity; thoracic duct cannulations also had indicated that peroxides were destroyed in the gut, though their reduced products were absorbed. However, the toxic event was not revealed. In any event, a peroxide value of 13 did not seem to be toxic to growth (Fig. 6B).

Fig. 6. Effects of Moderate Peroxide Values on Growth of Rats Under Special Circumstances

From Andrews et al. (029)

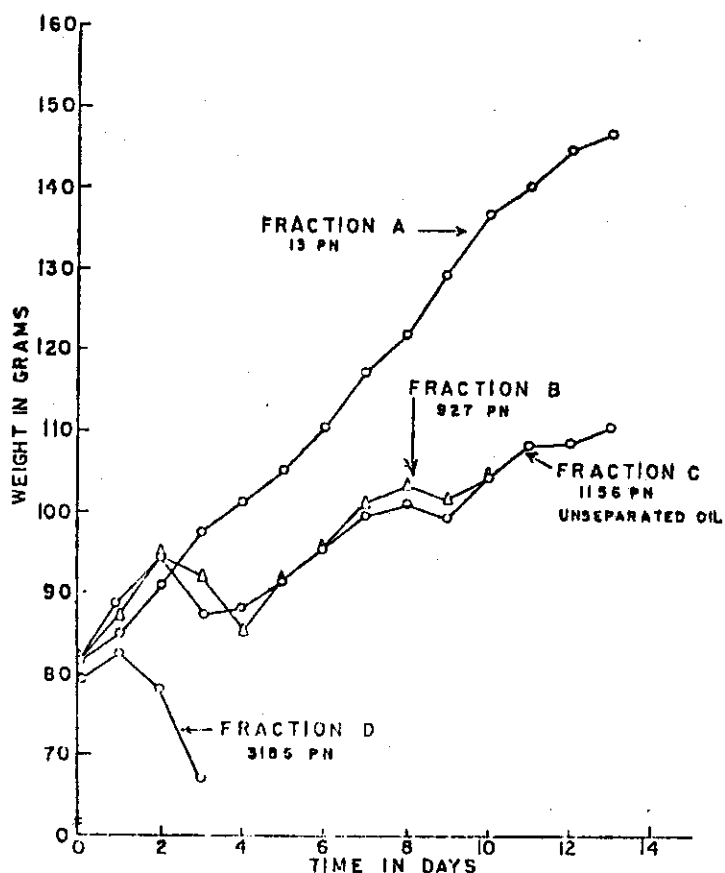
A. Soybean oil of peroxide value 100 fed as 15 percent of a diet during recovery from whole-body irradiation



Effect of diet on recovery from whole body irradiation

Fig. 6 (cont.)

B. Effect of fractions of oxidized soybean oil of various peroxide values



Growth of rats receiving various fractions of oxidized soybean oil

Roubal and Tappel (357) studied the in vitro effects of peroxidizing lipids, typified by ethyl arachidonate, on the integrity of proteins and amino acids. Measurable denaturation was found in six proteins. Five proteins were analyzed for damage to their amino acids, and seventeen of those were reported damaged. Causation of the damage was attributed to generation of transient free radicals. In addition, cysteine was part-converted to cystine with liberation of H_2S . The authors concluded that such damage "appears to be about

one-tenth as effective as radiation damage."

The pathway by which peroxidic free radicals were generated during autoxidation of linoleic acid was reported by Dam (099). The possibility that the cyclic monomer, isolated by Matsuo (286), may arise as an endproduct of peroxidation in hydrogenated soybean oil was implied by the author but has not been demonstrated in fact.

III. Long Term Studies

No reports on long term studies of chronic toxicity of hydrogenated or partly hydrogenated soybean oil have been found in the literature.

Nevertheless, there appears to be a theoretical possibility that epoxides might arise either during metabolism of peroxides in the intestine or by action of drug-metabolizing enzyme systems on soybean oil hydrocarbons (see Breakdown); there is a further possibility that such hydrocarbons might be carcinogenic. This could apply, of course, to virtually all edible oils if overheated during frying. Grover (158) has reviewed the mechanisms. Apparently epoxides that form at the K regions (regions of high electron density associated with isolated phenanthrenoid double bonds) or other regions of cyclic hydrocarbons are electrophilic. When these epoxides react with nucleic acids in vitro or in cultured cells, alkylation may occur; or alternatively, an epoxide may be intercalated into DNA or may delete a DNA base, and each case could result in a frameshift mutation. On reinjection into rodents, resultant morphologically abnormal cell colonies reportedly have been found to

be malignant.

None of these effects have been reported for partly hydrogenated soybean oil in vitro or in vivo. However, the rate of growth of carcinosarcoma in rats was reported by Haven (174) to be faster when the dietary fat was coconut oil of iodine number 157 than when it was cod liver oil of iodine number 9; the variation was attributed to the difference in saturation of the respective PUFA.

The current debate about the possible carcinogenicity of selenium may be indirectly relevant to hydrogenated soybean oil. As reviewed by Scott (367), Se has long been known to have a sparing effect upon vitamin E both in chicks and in second-generation rats. Se reportedly participates in the glutathione peroxidase reaction in rat erythrocytes. Reportedly, there is a "very high incidence of selenium deficiency in the eastern part of the United States." This deficiency is due to low Se content of animal feeds, including soybean meal, as well as to its low biological availability (65% average for soybean meal), resulting in losses among poultry and severe Se deficiencies in other livestock. A request to the Food and Drug Administration (FDA) from the American Food Manufacturers Association (AFMA) to permit supplementation of feeds with Se to a level of 0.25-0.35 ppm (dated 3.9.1970) has been delayed by uncertainty as to the status of Se under the Delaney Amendment. According to Scott, Se is an essential nutrient in the range 0.1-0.3 ppm in the total diet but produces chronic toxicity in the range 2-10 ppm. In the lower range, according to Scott, it has been shown to inhibit cancers, while "a carcinogenic effect of high

Table 11

FDA Tolerances for Pesticides in Oil Seeds in 1968,
And Amounts Added to Cottonseed Oil Before Its
Refinement in Order to Determine Their Persistence
[Adapted from Gooding (147)]

Pesticide	FDA Tolerance, ppm	Amount Added
Aldrin	0.25	1.0
Benzenehexachloride (BHC)	5.0	15.0
Chlordane	0.3	1.0
DDT	7.0	21.0
Dieldrin	0.1	1.0
Heptachlor	0.0	1.0
Heptachlor Epoxide-99.4%	0.0	1.0
Kelthane	0.1	1.0
Lindane	10.0	30.0
Methoxychlor	14.0	42.0
Sesone	6.0	18.0
Strobane	5.0	15.0
TDE or DDD	7.0	21.0
Toxaphene	7.0	21.0

residues in edible oils (as contrasted with seeds) and concluded that none were needed.

Stout (389) monitored food fish caught in the Northwest Pacific for levels of DDT and related pesticides, and found up to 0.4 ppm which she attributed to agricultural run-off.

Smith et al. (378) conducted a controlled study to see if Gooding's results could be repeated. They used, in all, five samples each of cottonseed and soybean oils. Levels of fortification were 1.0 ppm for endrin, DDE, aldrin, dieldrin, heptachlor, and heptachlor epoxide, and 21.0 ppm for DDT. Three laboratories participated, and methods were "essentially equivalent" to those prescribed by the FDA. For convenience the data are assembled in Table 12. Although these experiments were performed under pilot-plant rather than commercial conditions, the authors decided that the "preponderance of evidence" supported a conclusion that processed edible oils are free of chlorinated residues including endrin. They speculated that any residues surviving refinement would be volatilized and removed during hydrogenation, deodorization, or both.

Stout et al. (390) reported that all detectable amounts of p,p'-DDT could be removed from fish oils by refining and 90% could be removed by dehydrogenation; however, p,p'-DDE levels were substantially reduced but not eliminated. Reviewing their own and others' studies the authors concluded that oils, including vegetable oils, should be deodorized at 230°C or higher (pressure 6 mm Hg) to remove 95% of chlorinated pesticides, and that

Table 12

Removal of Chlorinated Pesticide Residues From
Samples of Cottonseed and Soybean Oils During
Refinement and Dehydrogenation

From Smith et al. (378)

A. Results Obtained in Processing the Vegetable Oils Under Pilot
Plant Conditions^a

Process	SBO-1	SBO-2	SBO-3	SBO-4	SBO-5	CSO-1	CSO-2	CSO-3	CSO-4	CSO-5
Refining										
Quantity of oil refined, lb.	222	226	210	232	295	225	212	221	203	295
Free fatty acids in oil, wt. %	0.71	0.72	1.17	0.30	0.30	2.70	2.75	2.60	2.20	0.49
Caustic used, lb.	12.6	19.2	20.4	16.9	22.5	21.6	20.8	21.0	17.9	23.2
Refined oil recovered, lb.	209	204	182	187	260	191	178	182	178	241
Refining loss, wt. %	5.9	9.7	13.3	15.8	11.9	15.1	16.0	18.4	12.3	18.8
Bleaching										
Quantity of oil bleached, lb.	206	201	179	184	255	188	175	179	175	286
Bleached oil recovered, lb.	197	200	176	175	245	187	168	176	162	225
Hydrogenation										
Quantity of oil hydrogenated, lb.		197		172						
Hydrogenated oil recovered, lb.		147		86						
Deodorization										
Quantity of oil deodorized, lb.	194	147	173	200 ^b	200	184	165	173	159	200
Deodorized oil recovered, lb.	189	136	170	198	190	172	156	166	144	186
Condensate recovered, lb.	0	0.25	0.25	4.0	4.0	3.75	0.5	0.25	0.25	0.25

^a SBO = Soybean oil.

CSO = Cottonseed oil.

^b Composed of 66 lb. hydrogenated SBO-4 and 114 lb. deodorized SBO-2.

B. Level of Endrin Detected in the Various Crude Vegetable Oil Lots

Oil sample	Location produced	Level endrin found (ppm) ^a			
		Lab 1	Lab 2	Lab 3	Average ^b
SBO-1	Area suspected of contamination	0.61	0.78	0.44	0.62
SBO-2	Area suspected of contamination	0.77	0.51	0.14	0.44
SBO-3	Area suspected of contamination	0.41	0.43	0.36	0.40
SBO-4	Area suspected of contamination	0.35	0.24	0.07	0.22
SBO-5	Area not suspected of contamination	BDL	BDL	0.03	0.01
CSO-1	Area suspected of contamination	BDL	0.03	BDL	0.01
CSO-2	Area suspected of contamination	BDL	0.19	BDL	0.06
CSO-3	Area suspected of contamination	BDL	BDL	0.03	0.01
CSO-4	Area suspected of contamination	I	BDL	BDL	BDL
CSO-5	Area not suspected of contamination	BDL	0.05	BDL	0.02

^a SBO = Soybean oil.

CSO = Cottonseed oil.

BDL = Below detectable limits of analysis.

I = Interference in sample analysis.

^b Calculated by taking average of values reported by the three laboratories.

Table 12 (cont.)

C. Effect of Processing on Endrin Residue Levels in Four Soybean Oils Initially Suspected of Pesticide Contamination^a

Oil sample	Crude oil	Refined oil	Bleached oil	Deodorized oil
		ppm		
SBO-1	0.62	0.59	0.51	BDL ^b
SBO-2	0.48	0.30	0.35	BDL
SBO-3	0.40	0.64	0.48	BDL
SBO-4	0.22	0.30	0.20	BDL
Mean	0.43	0.46	0.41	BDL

^a Individual crude, bleached and deodorized oil values represent mean analyses of the three laboratories. Two analyses compose the refined oil values.

^b BDL = Below detectable limits of analysis.

D. Pesticide Residues Found in Fortified Cottonseed Oils at Various Stages of Processing^a

	Refined oil	Bleached oil	Deodorized oil
	ppm		
Endrin	0.42	0.65	b
DDT	11.1	12.5	c
DDE	0.92	0.83	BDL ^d
Aldrin	0.55	0.65	BDL
Dieldrin	0.72	0.64	BDL
Heptachlor	0.58	0.69	BDL
Heptachlor epoxide	0.77	0.86	BDL

^a Average pesticide value of two oils with three independent laboratory analytical values per oil.

^b Values (CSO-1, 0.07; CSO-2, 0.08) were reported by one laboratory and the other two laboratories could not confirm the presence of a detectable level of residue.

^c A value of 0.06 ppm was reported for CSO-2 by one laboratory and not confirmed by other laboratory analyses.

^d BDL = Below detectable limits of analysis.

E. Average Endrin Content in Various Oil Fractions Obtained in Processing the Four Soybean Oils^a

	Total endrin content
	mg
Crude oil	43.1
Soapstock oil loss	4.8
Refined oil	40.9
Bleached oil	36.5
Deodorized oil	BDL ^b

^a Values are calculated from mean endrin analyses and oil fraction weights of the four soybean oils.

^b BDL = Below detectable limits of analysis.

refining, bleaching, and hydrogenation were relatively ineffective. They pointed to risks of thermal degradation and emphasized that deodorization should be prolonged when temperatures were restricted to the more usual 190-200°C.

Thus in the literature consulted during preparation of this monograph, there was disagreement about the efficacy of the usual processes of refinement, hydrogenation, and deodorization for removing all, or even substantially all, traces of chlorinated pesticide residues from edible vegetable oils, including partly hydrogenated soybean oil. The data show that some residues can persist, and it is a fact that no official tolerance levels have been set.

Less work has been reported on detection and removal of polychlorinated biphenyls (PCB), which can accidentally contaminate edible oils. Beezhold and Stout (043) have described a gas chromatographic method of detecting PCB in fishery products, but they report problems with analytical standards that are still being studied. No reports were found on elimination of PCB.

BIOCHEMICAL ASPECTS

I. Breakdown

Perhaps the first stage of breakdown is the formation of volatile by-products during hydrogenation. These give rise to the well-known "hardening flavor" and are largely removed during deodorization.

Kawada *et al.* (228) analyzed by gas chromatography a soybean oil, commercially refined and bleached, with iodine number 131 and peroxide value 10.5 meq/kg (contrasted with the usual 0.5-2.0 meq/kg). The results have already been given (Table 5 and Figure 3). The authors identified gamma-hexalactone (Fig. 3, parts C & D) by the logic illustrated in Figure 7. They stressed that volatile derivatives of hydroperoxides generated by hydrogenation differ in quality and quantity from those resulting from autooxidation.

Lea (252) studied the oxidation of lard in contact with an aqueous phase and found it to be more rapid at lower pH. He found that Cu accelerated oxidation when diluted to 0.01 ppm, and that Fe was about 5% as active. Antioxidants varied in their inhibitory effects, and Lea concluded that they did not function by antagonizing the pro-oxidant property of Cu; he thought that Cu might function by destroying natural antioxidants.

No detailed studies of autooxidizing hydrogenated soybean oil were found; some data on other oils are given to show the sorts of experiments that may be needed.

Wyatt and Day (424) analyzed autooxidizing salmon oil quantitatively for volatile carbonyl compounds (Table 13). No such list was found for autooxidizing hydrogenated soybean oil.

Figure 7

Identification of Gamma-Hexalactone By Mass Spectrum
and Its Derivation From Hydroperoxides of Hydrogenation

From Kawada et al. (228)

A. Interpretation of the mass spectrum of the gas chromatographic
fraction identified as γ -hexalactone

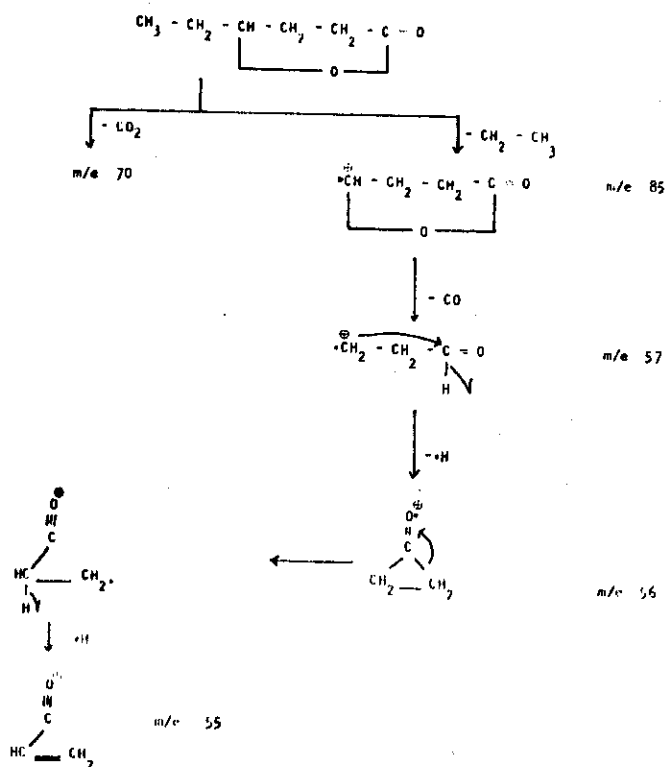
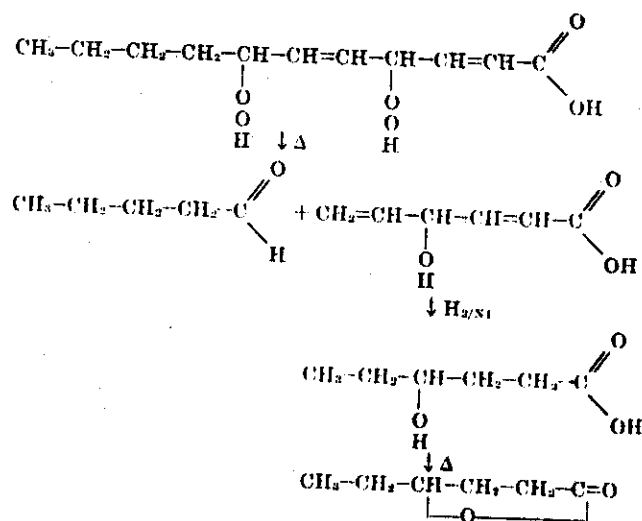


Figure 7 (cont.)

B. The lactones could only be formed through hydroxy acids. A mechanism for the formation of γ -hexalactone from the dihydroperoxide of a free fatty acid which may originate from linoleate is postulated as following:



C. A second mechanism involves the secondary autoxidation of 3-*cis*-hexenoic acid is also possible. This acid may be produced by the autoxidation of linolenate as follows:

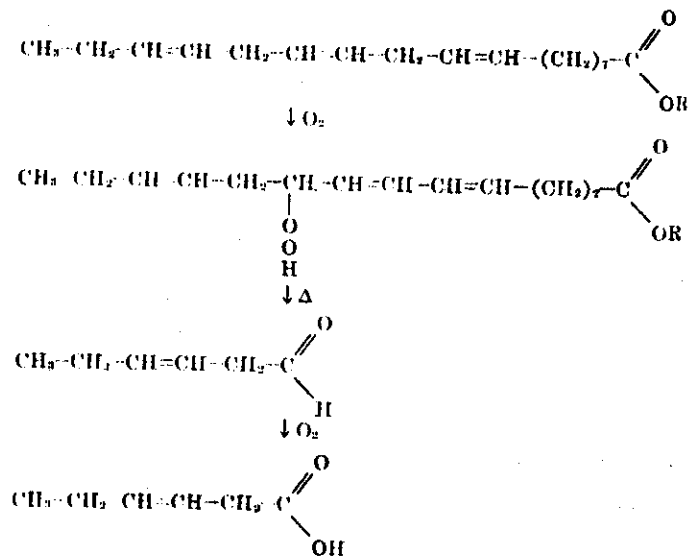
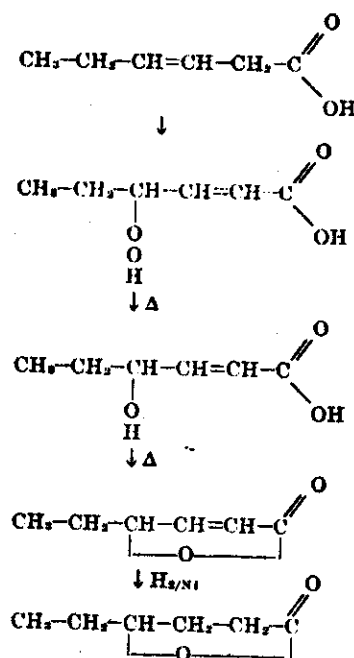


Fig. 7 (cont.)

D. This acid may form a hydroperoxide which is relatively nonvolatile and may remain in the oil during the deodorization previous to hydrogenation.



Chipault (082) found that the volatile carbonyls of oxidized fish oils included formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, valeraldehyde, hexaldehyde, acetone, butanone, pentanone, alpha-beta-unsaturated monocarbonyls, glyoxal, other low-mw alpha-keto aldehydes and diketones, and 2,4-dinitrophenylhydrazones not further identified. The basic fractions of the volatiles contained small amounts of amines, and the acidic fractions, only acetic and propionic acids.

Olcott (326) has summarized the biochemistry of autoxidation and antioxidants in oils, as shown in Figure 8. Steps (1)-(3) are "characteristic of free radical chain reactions" as to speed and

Table 13

Carbonyl Compounds in Autoxidizing Salmon Oil

From Wyatt and Day (424)

A. Gross changes in salmon oil during oxidation

	Sample no.				
	1	2	3	4	5
Days' storage	0	16	31	66	163
TBA no.	8.35	350	1102	2024	1472
Peroxide value	4.0	62.6	171	224	358
Total carbonyls ^a	9.5	52.6	110.8	312.3	569.8
Volatile monocarbonyls ^a	0.096	0.176	0.562	1.463	2.057
Girard-isolable ^a monocarbonyls	4.456	3.820	98.670	77.888	19.209
Volatile dicarbonyls ^a	0.039	0.047	0.289	0.308	0.890
Girard-isolable ^a dicarbonyls	0.194	1.114	3.309	5.972	8.250

^amM/kg of oil

Table 13 (cont.)

D. Volatile monocarbonyl compounds from autoxidizing salmon oil
(mM/kg).

	Sample no.				
	1	2	3	4	5
Alkanals					
n-Dodecanal	.0029	.00170126
n-Undecanal	.0023	.0022	.0058	.0709	.0194
n-Decanal	.0022	.0018	.0102	.0705	.0675
n-Nonanal0019	.0058	.1292	.0606
n-Octanal	.0015	.0016	.0087	.1212	.0720
n-Heptanal	.0020	.0023	.0109	.0758	.0755
n-Hexanal	.0024	.0043	.0310	.2234	.2549
n-Pentanal	.0025	.0097	.0358	.1794	.0269
n-Butanal0036	.0375	.0698	.0274
n-Propanal	.0079	.0421	.12871875
n-Ethanal	.0168	.0062	.06211600
n-Methanal	.0111	.0720	.13350514
Total	.0516	.1494	.4700	.9400	1.0517
Alk-2-enals					
Dodec-2-enal	.0008	.0006	.0010	.0031	.0407
Undec-2-enal	.0011	.0006	.0008	.0041	.0101
Dec-2-enal	.0016	.0015	.0018	.0139	.0275
Non-2-enal	.0010	.0010	.0025	.0291	.0407
Oct-2-enal	.0012	.0013	.0019	.0272	.0473
Hept-2-enal	.0023	.0022	.0025	.0595	.0891
Hex-2-enal	.0024	.0030	.0205	.0724	.2463
Pent-2-enal	.0054	.0034	.0262	.0451	.2386
But-2-enal0146
Total	.0159	.0135	.0717	.2543	.7412
Alk-2,4-dienals					
Dec-2,4-dienal	.0022	.0010	.0008	.0198	.0080
Non-2,4-dienal	.0012	.00040120	.0627
Oct-2,4-dienal	.0025	.0011	.0023	.0225	.0458
Hept-2,4-dienal	.0227	.0102	.0143	.1704	.1005
Hex-2,4-dienal0009	.0029	.0441	.0471
Total	.0285	.0135	.0204	.2688	.2641

Table 13 (cont.)

C. Volatile dicarbonyl compounds from autoxidizing salmon oil (mM/kg).

Fraction	Sample no.				
	1	2	3	4	5
1	.0019	.0150	.0742	.0213	.2211
2	.0345	.0065	.0550	.1455	.1982
3	.0027	.0098	.0631	.0923	.0734
40164	.0966	.0491	.3968

extent, and step (4) shows the formation of additional radicals during decomposition of the hydroperoxide. An antioxidant (AH) reacts with a free radical formed in the early stages to give an intermediate that is incapable of continuing the chain reaction. Naturally occurring antioxidants mainly are methyl derivatives of tocol, in which the side chain R_4 is either a hydrocarbon chain (C_{17}) or a triene group, as shown in the table at the bottom of Figure 8.

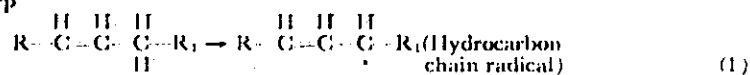
Figure 8

Typical Autoxidation and Action of Antioxidants

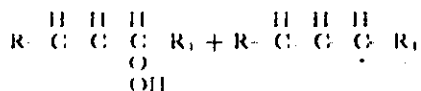
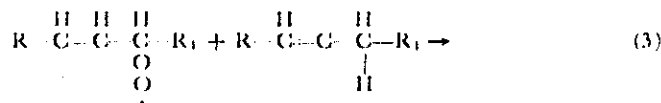
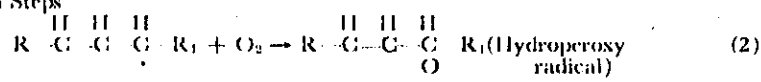
From Olcott (326)

A. Oxidation

Initiation Step



Propagation Steps



B. Antioxidant (AH) reactions

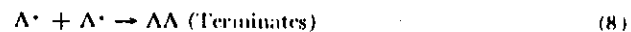
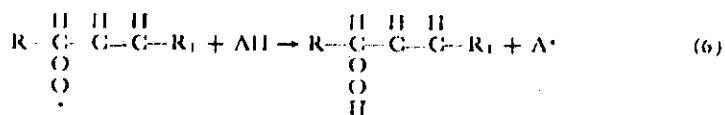
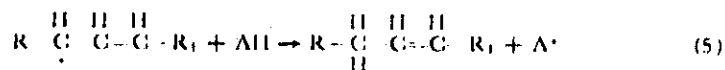
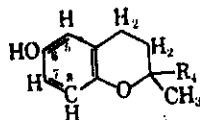


Figure 8 (cont.)

c. Tocol and some derivatives



Tocol

STRUCTURE AND RELATIVE ANTIOXIDANT ACTIVITY OF
TOCOPHEROLS IN SQUALENE (50°)¹

	Methyl Groups	Relative Antioxidant Activity
Tocols		
Alpha	5,7,8	1
Beta	5,8	5
Gamma	7,8	18
Delta	8	11
Tocotrienols		
Zeta	5,7,8	1
Epsilon	5,8	4
Eta	7,8	10

¹ R₄ chain of tocol is $-\text{CH}_2(\text{CH}_2-\overset{\text{CH}_3}{\underset{|}{\text{CH}}}-\text{CH}_2)_3\text{H}$. R₄ side chain
of tocotrienol is $-\text{CH}_2(\text{CH}_2-\text{CH}=\overset{\text{CH}_3}{\underset{|}{\text{C}}}-\text{CH}_2)_3\text{H}$.

As already mentioned, the reversion of hardening flavor in soybean oils is probably due partly to hydrogenation and oxidation of aldehydes to give 6-trans-nonenal and related compounds (385). Hannewijk has concluded that if hydrogenated oils are prepared carefully, supplementary antioxidants are not needed; if not, they are of little avail.

Hamberg and Samuelsson (164) studied the positional and stereochemical specificities of oxygenation of unsaturated FA by soybean lipoxidase. The reaction was found to be almost entirely specific, and the oxygen function was introduced at position ω -6 in all FA that reacted, except that in one sample a small amount of substrate was oxygenated at position ω -10. The requirements for substrates in this reaction were elucidated, and it appeared that hydrogen removal was the initial step of the reaction.

II. Absorption - Distribution

McCay and Paul (291) confirmed, in guinea pigs, that utilization of different fats including soybean oil diminished as the melting point rose. Their criterion was the proportion excreted in the feces. This conclusion implies, of course, that absorption of hydrogenated soybean oils will decrease as the iodine number diminishes, and may be as little as 60%.

Thomasson (397), using rats, divided 18 natural oils into five classes according to their rates of absorption but found, nevertheless, that the gut distribution was relatively constant at about 73%, 22%, and 5% in stomach, small intestine, and colon

respectively. Soybean oil, in the second fastest absorbed class, was relatively more abundant than average in the small intestine (28-38%); this, however, would not necessarily apply also to hydrogenated soybean oil.

Hartwell (173) studied the digestion of various fats by a semi-purified pancreatic lipase in vitro. She found that butter was digested more rapidly than other fats except for coconut, palm kernel and castor oils, and that soybean and hardened arachis oils were in the slower group that included animal fats and vegetable oils.

These studies together suggest that the mechanisms of digestion and absorption of hydrogenated soybean oil are the same as those for other fats regarded as major nutrients.

It is well known that fatty acids first synthesized in plants can persist through the food chain. Beare (042) fed rats a purified diet containing 20% w/w of corn oil, hydrogenated herring oil, or margarine, and measured the FA distribution in liver, carcass, and milk fats. She found that the distribution of palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, eicosanoic, eicosadienoic, and erucic acids in milk fat reflected their pattern in the diet, generally more closely than did their carcass distribution. Tissue levels of arachidonic acid, not provided in the diet, were related to dietary linoleic acid levels. Changes of dietary fat were followed by corresponding changes in tissue distribution of long-chain FA. Beare observed that the rats required a long time to adapt to utilization of erucic acid.

Mohrhauer and Holman (309) fed rats supplements of purified FA

(ethyl linoleate, ethyl arachidonate, or ethyl linolenate) at 0.1-10% of the calories, and measured the FA composition of the liver lipids. Linoleate over 1% was increasingly stored and converted into FA of the linoleate family (20:4 and 22:4 acids). Arachidonate also was stored increasingly and converted to 22:5- ω -6. Linolenic acid was stored and converted to 20:5, 22:5- ω -3, and 22:6; also the level of 20:4 was decreased. There was, however, no conversion to linolenate family from linoleate or arachidonate. All three FA diminished the level of 20:3.

Aaes-Jørgensen (004) reviewed the distribution of marker FA through the tissues, radiating from the mesentery, but did not include a discussion of soybean oil.

Imaichi et al. (200) used gas-liquid chromatography to study the FA composition of 210 samples of human depot fat removed at surgery from abdominal or thoracic walls. Ten of these patients were known to be high consumers of fish oils, and their depot fat contained twice the average levels of highly unsaturated long-chain PUFA. The authors also noted some sex differences in the distribution of palmitoleate, myristoleate, and stearate.

Hølmer and Aaes-Jørgensen (189) fed rats one or another of four oils as sole dietary fat; these were arachis oil, or partly hydrogenated oils of arachis, soybean or herring, with distinctive FA patterns, and they were given as 28% w/w of the basal diet. The distribution of neutral lipids and phospholipids in the testes was measured after 5, 15 and 26 weeks and was found to be consistent with tissue degeneration reported separately by the same authors (008)-- see Short Term Studies.

leate to arachidonate was progressively inhibited by increasing amounts of linolenate in the diet.

Menzel and Olcott (297) studied the positional distribution of FA in various animal lecithins in vitro, and concluded that linoleic and arachidonic acids are probably interchangeable with "non-essential" polyenoic acids with regard to some biochemical reactions in metabolism.

Holman (188) reviewed the interrelationships between FA in metabolism and found them extensive, but emphasized that ω -3 and ω -6 acids were not interconverted. He proposed a system for chain elongation during metabolism, shown in Figure 9, which reflects competitive inhibitions of the type described by Mohrhauer and Holman (310). Thus the metabolism of PUFA would depend on the contents of the FA pool in the body, as well as on the distribution of PUFA in the diet; a balanced diet would take all of this into account.

These findings were pursued in greater detail by Brenner and Peluffo (264) and by Mohrhauer et al. (311), with results that are important to knowledge of FA metabolism in general, rather than to partly hydrogenated soybean oil in particular.

The situation with maximally hydrogenated oils appears, however, to be different. Paluszak (333) hydrogenated a sample of rapeseed oil to mp 59°C and iodine number 5, eliminating linoleate, linolenate, and arachidonate. He fed this as a diet supplement to young rats, with and without added cholesterol and biotin (1% and 2 ppm respectively), for 11 weeks. Losses of weight and disappearance of depot fat were attributed to deficiencies of EFA activity and of biotin associated with the extreme hydrogenation.

Horst et al. (193,194) fed rats a diet with 50% w/w soybean oil partly or maximally hydrogenated (iodine numbers 130, 62, and 9). At iodine number 9, linoleic acid disappeared from adipose tissue and total body fat; depot fat also disappeared and did not return after the rats had been transferred to basal diet for 10 weeks. The oil had been hydrogenated at 200°C, and the authors looked for thermal and isomerization effects. But, "toxic effect was not found in our rats. They seemed to be well and had a normal and healthy appearance despite their progressive loss of body weight." The authors suspected a block in endogenous FA synthesis from C₂ intermediates of the Krebs cycle, suggesting that maximally hydrogenated fats might inhibit lipogenesis from carbohydrates, and that this ought to be studied.

It is well known that a major route of excretion of lipids is the sweat. However, most fats are utilized by the body as energy sources, or are stored when food intakes are persistently excessive.

IV. Effects on Enzymes and Other Biochemical Parameters

As Horst and his coworkers (193,194) had suspected, and as already noted in the foregoing discussion of Vitamin E or Antioxidant Deficiency (Short Term Studies), the nature of the basal diet can greatly influence the observed metabolism, especially the presence of simple and readily soluble carbohydrates.

Williams and Carroll (420) designed some complicated experiments with rats to elucidate metabolic interactions between dietary proteins, carbohydrates, and fats. For carbohydrates, they used glucose or fructose; for fats, regular or high-oleic vegetable oils; and

proteins were selected of different amino acid compositions. Some of the diets were categorized as "imbalanced". At the end of each experiment, the amounts and FA compositions of body lipid accumulations were determined. In general the FA compositions of body lipids were found to reflect those of the oils fed. All of the "imbalanced" diets altered the FA compositions of liver and adipose tissue lipids so that the proportion of 18:0 was diminished, those of 16:1 and 18:1 were increased, and the ratio of 18:2/20:4 was increased. Fructose accentuated both the increases and the decreases from normal.

Thus, metabolic studies in animals receiving "basal" diets of casein and sucrose in ratios such as 20:70 w/w, supplemented with relatively small amounts of different oils, are of limited value for determining the metabolism of the oils or the effects of the oils on other metabolic processes. Such studies can reveal only what happens in the presence of massive lipogenesis from carbohydrate. The FA compositions of the oils were reflected in the body lipids, and the decreases and increases were accentuated by fructose.

Sassoon et al. (360) demonstrated (as have others) the extent to which diets high in soluble carbohydrate can induce increases of glycolytic pathway enzymes. Also, they elucidated part of the mechanisms of induction. Dror et al. (117) have shown that this induction disturbs the pattern of protein utilization in rats, causing a 35% loss of efficiency judged by nitrogen balances. Reviewing the time course of metabolism in the presence of high-soluble-carbohydrate diets, Sassoon (359) has suggested that enzymatic adaptations to glycolysis, rather than to gluconeogenesis from lipids,

can overwhelm many physiological processes; this is especially so with sucrose diets because fructose tends to evade the normal glycolytic pathways and to be converted almost directly to glyceraldehyde-3-phosphate.

Thus, studies in animals receiving "basal" diets of casein: sucrose 20:70 w/w, or thereabouts, that are supplemented with relatively small amounts of oils (part-substituted in many cases) can reveal only what happens to or because of the oils in the presence of massive lipogenesis.

Egwin and Sgoutas (120) fed rats a basal diet of casein and sucrose, 16:74% w/w, and for some groups they partly replaced sucrose isocalorically with 10% corn oil, 20% corn oil, or 20% partly hydrogenated soybean oil. Each oil characteristically altered the activities of acetyl-CoA carboxylase and FA synthetase in both liver and adipose tissues. The mechanisms of these effects were not identified, and without taking into account the lipogenicity of the basal diet, the authors speculated that the effects might be related to EFA activity and trans-FA content of the oil supplements.

Andrews et al. (129) added fresh or air-oxidized soybean oil, not hydrogenated, to a basal casein-sucrose diet, 18:61% w/w, fed to rats, and found that the activity of intestinal xanthine oxidase was lower when the diet contained the oxidized soybean oil. The authors concluded that the enzyme was inhibited by the oxidized oil, but they did not test for the decrease except in the presence of the sucrose. However, they did find that both milk xanthine oxidase and the rat intestinal enzyme were inhibited in vitro by t-butyl hydroperoxide.

These findings, therefore, may be regarded as suggestive but incomplete.

Booth et al. (061) reported that when various vegetable oils were added to a control diet fed to female mice, the mean weight of the uterus increased by varying amounts; it was nearly doubled by refined soybean oil. They described this activity as "estrogen-like," and implied that estrogens were among the lipids in the oils; however, they supplied no analytical evidence.

Mecchi et al. (293) fed chickens and turkeys a basal diet in which the source of the fats was not stated, and found that supplementation with 0.1% tocopherol "increased the tocopherol content and stability (induction period) of carcass fat." They confirmed this by analyses after the carcasses had been stored for nine months at +10°F.

Van der Steur (412) reviewed the effects of refining and hydrogenation on the stability of additives including the fat-soluble vitamins. He noted that little of vitamin E was lost, but much of vitamins A and D is; he cautioned, however, against over-replacement.

Kishimoto et al. (238) analyzed slices of the left cerebral hemispheres of patients who died of multiple sclerosis and found subnormal axon densities and ganglioside concentrations in the plaques; they also observed a FA composition of the plaques that resembled that of gray matter rather than that of the surrounding white matter. The composition of the surrounding white matter resembled that of control white matter from autopsies on healthy subjects.

Bernsohn and Stephanides (049) developed a theory that multiple sclerosis partly reflected demyelination resulting from dietary deficiency of ω -3 PUFA during the period of brain maturation. They associated the geographical distribution of diets poor in ω -3 PUFA with that of multiple sclerosis, noting that the disease was rare where soybean consumption was high. Fresh soybean oil is, of course, an excellent source of ω -3 PUFA, but this may not be so with some hydrogenated oils (see earlier discussion, e.g. Table 10).

Finally, a number of reports associate soybean oils or FA found especially in soybean oils with alterations of cholesterol concentrations in various body tissues.

Dam et al. (105) fed hamsters butterfat or a dietetic margarine containing about 40% linoleate (source not stated). After 6-7 weeks the hamsters were dissected; those in the margarine group had fewer cholesterol gallstones than those in the butter group. Where the fats were fed as 10% of the diet, the difference was highly significant ($P < 0.01$) and, where 3% of the diet, the difference was significant ($P < 0.05$).

Grollman (157) reported that of a large number of refined oils only some decreased blood pressure in animals with experimental hypertension. These included four fish oils and tung oil, but not soybean or other vegetable and land animal oils and fats that were tried. The site of action was presumed to be the kidney, and the active fraction of the oil was partly isolated but not identified.

Horlick (191) gave healthy students a fat-depleted diet supple-

mented with corn oil or one of two hydrogenated soybean oils (iodine numbers 40 and 75). Addition of the corn oil did not decrease the serum cholesterol level below that from the basal diet, but both soybean oils did; however, they were poorly absorbed and produced steatorrhea.

Zemplenyi (428) found that soybean oil (not hydrogenated) had no effect on development or regression of aortic atheromata of rabbits, or on related enzyme activities such as alkaline phosphatase, compared with control animals.

Horst et al. (194) fed rats a basal diet without sugars but with 25% of starch; other groups received this with equal amounts (w/w) of soybean oil hydrogenated to iodine numbers 130, 62, or 9 together with 1% of cholesterol. Serum lipids were elevated with oils of I numbers 130 and 62, and serum and liver cholesterol were elevated with oil of I number 130. There was no linoleate in the total body fat or adipose tissue of rats fed oil of I number 9.

Erickson et al. (124) fed seven diets, one of which was supplemented with partly hydrogenated soybean oil, to a total of 42 healthy men and administered 742 mg per day (average) of cholesterol to some of them; this raised the plasma cholesterol levels by an average 24-27 mg per 100 ml. The plasma cholesterol response to the type of fat fed was unaffected by hydrogenation, with or without the cholesterol supplement; neither did the plasma cholesterol level differ according to the dietary ratio of PUFA to saturated fats. Phospholipid levels in the plasma were also higher when the diet contained cholesterol, and were highest in the group that

received cocobutter as fat.

It is well known, of course, that the cholesterol/phospholipid ratio in the plasma is considered by many to be more important than the cholesterol level alone.

A Research Committee of 16 scientists (351) undertook a "controlled" trial of soybean oil (not hydrogenated) on 393 male patients who had recovered from their first myocardial infarction at hospitals in the region of London, England. Of two groups, one was given a diet low in saturated fats and supplemented with 85 g of soybean oil per day; individuals in the other ate their normal diets. The trial went on for nearly seven years; the shortest individual duration was two years, and the median about four years. Over a period of six months the serum cholesterol level of the soybean oil group fell an average 22%, and of the "free" diet group, 6%. There was no significant difference between the groups in the myocardial relapse rate. Relapses were unrelated to cholesterol levels, and the relapse rate was unaffected by the PUFA content of the diet.

Publication of these results was followed by letters of disappointment from Sinclair (372) and Gilder (143).

Stansby (384) reviewed the cholesterol depressant activities of oils from the standpoint of fish oils, which appeared to be more potent than vegetable oils. He concluded that the iodine number was "quite unreliable" as an indicator of cholesterol-depressant activity, and that degrees of "saturation" or "unsaturation" had little meaning because of the great differences in FA

structure and composition that were not revealed by this classification.

Kritchevsky et al. (247) fed young male rabbits a variety of vegetable oils, not including soybean oil, additionally 2% of cholesterol, for eight weeks. They then examined the aortas biochemically and histologically. Under those conditions the authors concluded that arachidic and behenic acids were responsible for the greater part of the atherogenesis observed, but acknowledged that other factors such as triglyceride structure might have had an important role.

V. Drug Interactions

No reports were found relating to soybean oil and drug interactions. It should be noted that fat-soluble substances used as drugs will become dissolved in soybean oils as in other oils, and if hydrogenated oil is used, they will be absorbed to the extent that the oil is absorbed; for example, if only 65% of a highly hydrogenated oil is absorbed, only that percentage of the drug given will probably be utilized.

VI. Consumer Exposure

Statistics are shown in Table 14-15. No separate statistics were found for hydrogenated soybean oils.

As of 1971 the major use of soybean oil was in shortenings (Table 14), and it can be presumed that most of this was hydrogenated. However, as already noted (see Description) the degree and processes of hydrogenation are by no means uniform.

Equally variable are the products described as soybean oil that are incorporated into prepared foods (Table 15), and their contributions to the lipid contents of those foods. Examples of this may be seen in Melnick et al. (295), and Bisno (054).

The second major use of soybean oils is in margarine (Table 14). The FDA Standard of Identity for Oleomargarine or Margarine (030) permits the use of partly hydrogenated soybean oil, and currently margarines contain about 75% of these oils. Overseas the recommended standard (084) is somewhat wider than in the United States, and margarines contain less of hydrogenated soybean oils. Nevertheless the FAO Books In Print (136) lists no standards for hydrogenated oils as such. Therefore, the exposure of consumers to hydrogenated soybean oils, whether in the United States or elsewhere, is unknown.

In short, no information was found on consumer exposure to hydrogenated soybean oil as such, whether alone or in combination with other dietary fats, and the only certainty (assuming the statistics to be reliable) is that it can not be more than the maximum overall production and intake data for all soybean oils (Tables 14, 15).

Table 14

Production and Overall Use of Soybean Oils

	1971	1970	1949
World production, millions tons:			
Vegetable, animal, marine oils	41.373	39.236	
Vegetable oils	20.505	19.545	
Soy oil	6.155	5.960	
USA total of fats and oils, millions lbs:			
Food use	11,009	11,161	6,419
Non-food use	5,383	5,675	3,735
Millions acres under soybeans:			
World	74.617		
USA	43.176*		
China	19.768		
Brazil	4.568		
USSR (no other country over 2 million acres)	2.162		
USA soybean oil production:			
Millions bushels soybeans crushed		761.265	
Millions lbs oil produced†		8,261*	1,937
Shipments and exports, millions lbs		1,782*	
Exports from USA, millions lbs (approximate):			
to N. America		147	
to S. America		219	
to Europe		305	
to Africa		211	
to Asia		870	
to Oceania		8.5	
Imports into USA of soybean oil, all from Denmark:			
thousands of lbs (1969 46,087 lbs; 1965 Nil)	37.452		
Soybean oil prices per lb: crude, in USA Midwest			
edible, in tanks at New York		12.9¢	12.3¢
		15.6¢	16.6¢

* A record amount

* Yield 27.6 bushels/acre*, contrasted with 13.3 bushels/acre in 1929.

+ The level of stocks in 1970 was 543 million lbs, comparable to other years.

Table 14 (cont.)

Production and Overall Use of Soybean Oils

	1971	1970	1949
USA margarine production, millions lbs:			
Total	1,831	1,794	701
Soybean oil content**	1,385	1,410	257
USA shortening production, millions lbs:			
Total	3,479	3,599	1,494
Soybean oil content***	2,047	2,182	713

** Cottonseed oil is the main other ingredient, e.g., 431 million lbs in 1949.

*** Other ingredients are listed as including lard, beef fat, and negligible quantities of fish oils.

These data, except for imports in USA, are abstracted from Agricultural Statistics, 1972, US Government Printing Office, Washington, D.C. The data on imports are abstracted from U.S. Imports for Consumption and General Imports, US Department of Commerce, Bureau of the Census, publications FT 246-71, FT 246-69, and FT 246-65, US Government Printing Office, Washington, D.C.

Table 15

Incorporation of Soybean Oils in Particular Foods
And Average USA Daily Intakes

A. Extracts from Comprehensive GRAS Survey, 1972, NAS NRC,
Washington, D.C.

Usage levels of soybean oil reported by industry (from Table 5)

Food category No. Name	Percent use, weighted means:	
	Usual	Maximum
01 Baked goods (R)	4.8	4.8
03 Other grains (R)	1.0	1.0
04 Fats and oils (R)	60.5	62.5
14 Processed vegetable (R)	0.5	0.9
25 Nut products (R)	9.0	11.0
30 Hard candy (R)	0.2	0.2
83 Formulas (B)	4.1	4.4

R = regular food, B = baby food.

B. Importance of soybean oil for technical effects and/or flavor,
as rated by industry (Table 9)

Use category No. Name	Percent importance rating:		
	A	B	C
05 Dough conditioner	100	0	0
07 Emulsifier	66.7	0	33.3
13 Formula aid	0	100	0
19 Nutrient supply	50	0	50
30 Texturizer	0	100	0
73 Hard candy	0	0	0

Ratings: A = "essential", e.g., flavor or effect unobtainable
with any other substance than soybean oil.

B = "important", e.g., alternatives either inordinately
expensive or give inferior results.

C = "useful", e.g., soybean oil is replaceable with
alternatives.

"Percent" is either the percent of situations in which soybean oil
is currently used, or the percent of soybean oil in the mixtures
currently used for the purposes described.

Table 15 (cont.)

C. Potential daily intakes, in milligrams of soybean oil, by age-cohorts of the US population, from various food categories (Table 15)

Food category No. Name	Age cohorts:			
	0-5 months	6-11 months	12-23 months	2-65 years
03 Other grains (R)	89 186	181 415	199 397	342 673
04 Fats and oils (R)	2,965 8,088	2,481 5,188	3,933 7,563	10,769 19,875
14 Processed vegetables (R)	41 138	151 517	202 578	428 1,262
25 Nut products (R)	36 55	999 2,385	522 1,485	981 2,607
30 Hard candy (R)	0 0	1 2	3 6	7 15
83 Formulas (B)	16,329 29,003	11,838 27,088	14,538 33,042	0 0
Calculated totals of above	19,460 38,070	15,651 35,595	19,397 43,071	12,527 24,432

Two estimates are given at each plot - "usual" and "very high", derived as follows:

"Usual" = average usage level in food, mean food consumption estimates.

"Very high" = maximal usage level in food, maximal food consumption estimates.

Two intermediate values are given in Table 15 - "High A" or maximal usage level, mean consumption; and "High B" or average usage level, maximal consumption. Low or minimal levels are not given in Table 15 or in other Tables in this survey. Thus the figures given above show the range from "average" actual intakes to maximal potential intakes, as estimated by the food industry.

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